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SECOND ANNUAL PROGRESS REPORT

TO NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

RADIOISOTOPIC BIOCHEMICAL PROBE FOR EXTRATERRESTRIAL LIFE

CONTRACT NASr-10

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I. SUMMARY

Dr. Norman Horowitz, Professor of Biology, California Institute of Technology, has become associated with the Radioisotopic Biochemical Probe for Extraterrestrial Life as an experimenter.

Extensive laboratory investigations and field testing have been carried out over the past year. These tests have further substantiated the feasibility of the principle on which Gulliver, the name now given to the Radioisotopic Biochemical Probe for Extraterrestrial Life, is based. Two major lines of investigation, biology and instrumentation, have been pursued concurrently and interdependently.

Several alterations of the basal medium have been made in which complex constituents such as the yeast extract and peptone have been decreased in concentration or omitted completely. Results indicate that dilution of these complex constituents is beneficial. A basal medium containing only K_2HPO_4, KNO_3, $MgSO_4 \cdot 7H_2O$, NaCl, and radioactive substrates has proven to be extremely satisfactory for obtaining responses from soil inocula.

Of 26 cultures of stock test organisms investigated (some during the preceding year), 22 have responded within 3.5 hours, three have responded within six hours, and one required longer than six hours. In addition, positive responses have been obtained from approximately 100 soil inocula. These included some garden soils, field soils, desert soils, as well as soils obtained with the instrument during field testing. No soil tested in the laboratory has ever failed to yield a positive response.

Several radioactive substrates have been evaluated in addition to formate and glucose. A beneficial effect resulted from the use of

DL-sodium lactate-1-C^{14}. No particular value has been obtained from aspartic or glutamic acids.

As a result of screening a number of chemicals for use as antimetabolites, only Bard-Parker Germicide (isopropanol, methanol, formaldehyde, and hexachlorophene) was found to be stable enough to heat, chemically unreactive with the labeled substrates, and still inhibitory to most of the organisms against which it was screened. It has been tested against soil inocula and has been found to be effective in reducing metabolic CO_2 levels. It has also been incorporated into field test controls with some success. However, the search for more effective inhibitors will be continued with the purpose of finding a completely effective means of inhibiting metabolism in the Gulliver control.

A third model of the instrument, designated Gulliver III, has been designed, fabricated, and successfully field tested. Gulliver III has omnidirectional capability, a chamber for carrying an antimetabolite and injecting it into the culture chamber, and a continued capability for flushing.

Improvements have been made in the method of deploying sample collection lines, in maintaining a constant temperature within the culture chamber, in types of soil sampling materials, and in flushing techniques.

Evaluation of methods for achieving maximum sensitivity is continuing.

Duplicate units of the third model of the instrument have been field tested successfully. Each unit was programmed identically, with the exception that an antimetabolite was automatically injected into one instrument shortly after a metabolic response was detected. Metabolic production of $C^{14}O_2$ continued in the uninhibited instrument but diminished in the inhibited test chamber. This type of test is a control, indicating the metabolic nature of the response obtained from the test unit.

Laboratory efforts have continued to define the nature of, and attempt to control the evolution of, nonmetabolic $C^{14}O_2$ from sterile media.

Experiments have been performed which show the feasibility of using the Gulliver, modified as a light-dark chamber, to investigate photosynthesis. A program in which Gulliver is used in a photosynthetic experiment is continuing.

II. BIOLOGICAL INVESTIGATION

Efforts have been made during the past year to define and improve the capabilities of the radioisotopic biochemical probe in each of the areas originally proposed. These included a study to improve the basal medium, an examination of new substrates, and refinement of various concentrations and specific activity levels of the substrates previously used. Antimetabolites for use in the control chamber were also to be investigated. Included in the biological portion of the investigation was a re-examination of the number and kinds of test organims in pure culture and an extension of the study of soils from various locations. Psychrophiles were to be included and an examination of the effects of temperature on CO_2 evolution was to be made. An investigation of the effects of heat sterilization on the medium was also proposed. The feasibility of adapting the radioisotopic probe to a photosynthetic experiment and of using tritium in the present experiment were to be considered. It was planned to field test the instrument and make modifications as needed.

All of the above aspects of the proposed program have been pursued. Progress has been made in each phase of the program although the degree of success has been greater in some areas than in others.

No changes in principle have been made in "Gulliver", the name now given to this approach for detecting extraterrestrial life.

A significant improvement was made in the method used for obtaining data in the laboratory during the past year. An automatic monitoring and recording system was developed which provides, (1) long term determinations with constant monitoring of $C^{14}O_2$, (2) simultaneous comparison of eight units, and (3) acquisition of data in a manner nearly identical to the system used in the space instrument. The unit consists of eight chambers which simulate the culture chamber of the second model of the instrument. The chambers are separated from Geiger-Müller tubes by a solid, $Ba(OH)_2$-coated, aluminized Mylar sheet acting as a CO_2 collector. The tubes are connected through a logarithmic count-rate meter to a multichannel recorder containing three cycle, semi-log paper. Medium in the chambers is inoculated with pure cultures or soil, and a continuous record is obtained showing the $C^{14}O_2$ evolution as a function of time. The entire unit, assembled by personnel from American Machine and Foundry Company, is shown in Figure 1.

The planchet method also continues to be utilized for certain determinations.

A. TEST ORGANISMS

1. Pure Cultures

Twenty-six organisms used during various phases of the testing program were listed, with pertinent characteristics of each, in the First Annual Progress Report. No additional organisms have been added to the collection, but testing of those not previously tested was accomplished during the current year. The responses obtained in M5 medium (see below) with sodium formate-C^{14} and glucose-C^{14} as the labeled substrates, are shown in Table 1. Because most of the organisms were tested many times, the responses listed are characteristic of those obtained routinely. Of the 26 cultures tested, 22 have responded within 3.5 hours, three have responded within six hours, and one required longer than six hours. The least responsive cultures have been the *Thiobacilli*.

Figure II-1. Entire Automated Monitoring Unit

TABLE II -1

ORGANISMS EVOLVING $C^{14}O_2$ WHEN TESTED IN M5 MEDIUM

Organism	CPM	Organism	CPM
Response Within 3.5 Hours			
Arthrobacter simplex	1,629	Mycobacterium phlei	1,913
Azotobacter agilis	28,956	Pseudomonas delphinii	971
Azotobacter indicus	1,868	Pseudomonas fluorescens	6,701
Bacillus subtilis spores	11,784	Pseudomonas maculicola	16,266
Bacterium bibulum	7,221	Rhodopseudomonas capsulata	365
Chlorella sp.	323	Rhodospirillum rubrum	420
Clostridium pasteurianum	1,698	Saccharomyces cerevisiae	3,093
Clostridium roseum	5,367	Staphylococcus epidermidis	3,219
Clostridium sporogenes	664	Streptomyces fradiae	443
Escherichia coli	65,389	Xanthomonas beticola	58,189
Micrococcus cinnabareus	840	Xanthomonas campestris	537
Response Within 6 Hours			
Photobacterium phosphoreum	2,423		
Thiobacillus novellus	141		
Thiobacillus thiooxidans	102		
Response Between 6 and 24 Hours			
Rhizobium leguminosarium	1,123		

Radioactive substrates: glucose-C^{14} (u.l.) and sodium formate-C^{14}.

Several changes have been made in the basal medium since the responses shown in Table 1 were elicited, but only a small number of representative test cultures have as yet been re-examined in this medium. The others will be tested in the modified medium during the next phase of the program.

2. Soils

Testing of soils is an important phase of the program. The heterogeneous population and the differences in the environments supply natural variables of considerable significance to the evaluation of the experiment and the instrumentation. In terms of extraterrestrial environments, it must be remembered, however, that earth soil has access to ample oxygen which will impose a bias favoring aerobic metabolism. Soil samples collected locally and from desert areas (provided by JPL) have been used. Incubation of the soils has been carried out at room temperature in planchets, and in the automatic monitoring system. The size of inoculum has varied from 10 to 100 mg per determination. Approximately 80 determinations (excluding instrumented field tests) using soils from nine different locations yielded positive responses without exception. No soil sample has been negative. Some representative data are presented in Table 2. As a generalization, samples collected at the surface yielded a higher response in a shorter time than subsurface soils. There were also several determinations made on soils incubated at 8°C with responses, obtained within 30 minutes, of approximately 500 cpm from one 10 mg sample and 1,400 cpm from a second 10 mg sample.

Sterilized soils have been tested periodically and have yielded no increase in nonmetabolic CO_2 levels above the control levels of the medium itself.

TABLE II-2

EVOLUTION OF $C^{14}O_2$ FROM VARIOUS SOILS INCUBATED IN PLANCHETS

Test Sample	Quantity Used (mg)	Time Elapsed After Inoculation (min)	Radioactivity Less Sterile Control (cpm)
Desert Soils			
#1	25	45	4,083
#75	25	45	1,478
#1	50	45	3,450
#1	50	180	9,100
#74	50	45	336
#74	50	180	1,140
#75	50	45	4,760
#75	50	180	11,800
#1	100	210	5,725
#74	100	210	2,799
#75	100	210	5,099
Garden Soils			
A	100	45	6,747
B	100	45	7,132
C	100	45	1,040
Field Soils			
	10	30	3,802
	25	45	4,000
		180	8,750
	50	45	5,000
		180	8,150
	100	45	5,550
	100	180	10,300

Data are taken from many experiments carried out under various conditions and in different media.

A representative response curve from soil incubated in the automatic monitoring unit is shown in Figure 2.

B. MEDIUM DEVELOPMENT

Throughout most of the past year, the basal medium used was the one previously reported as M5. Modifications have been made, primarily for the purpose of diluting or eliminating complex constituents from the medium in order to prevent inhibitory effects on some organisms. The composition of the media and identification numbers are listed below:

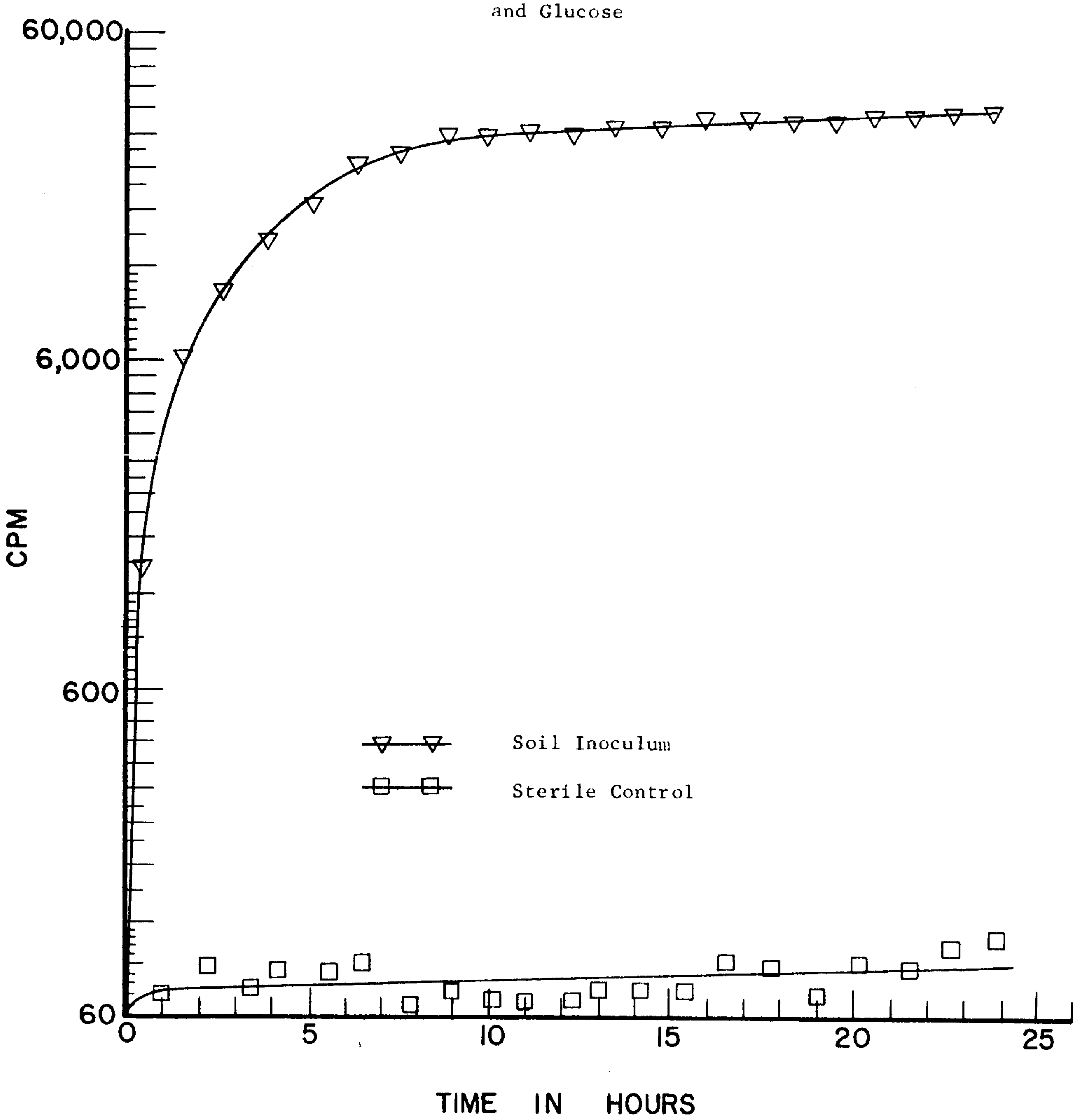
Constituent	Medium Number M5	M6	M7	M8
K_2HPO_4	1.0 g	1.0 g	1.0 g	1.0 g
KNO_3	0.5 g	0.5 g	0.5 g	0.5 g
$MgSO_4 \cdot 7H_2O$	0.2 g	0.2 g	0.2 g	0.2 g
NaCl	0.1 g	0.1 g	0.1 g	0.1 g
$FeCl_3$	0.01g	0.01g	0.01g	-
Soil Extract	250.0 ml	250.0ml	250.0 ml	-
Malt Extract	3.0 g	3.0 g	3.0 g	-
Ascorbic Acid	0.2 g	0.2 g	0.2 g	-
Beef Extract	3.0 g	3.0 g	3.0 g	-
L-Cystine	0.7 g	0.7 g	0.7 g	-
Na_2SO_3	0.2 g	-	-	-
Bacto Casamino Acid	4.0 g	4.0 g	-	-
Yeast Extract	13.0 g	6.5 g	6.5 g	-
Proteose Peptone #3	20.0 g	10.0 g	10.0 g	-
Distilled H_2O		up to 1 liter		

1. Dilution of Complex Constituents

In a series of studies using pure cultures or soils as inocula, the yeast extract and peptone in M5 were diluted to 0.25% and 0.5% of their

FIGURE II-2

Evolution of $C^{14}O_2$ From 100 Mg Sample of Garden Soil Incubated in an Aqueous Solution of Radioactive Formate and Glucose



original concentrations. Although they were variable, in general, the responses from the dilutions were better than from the regular M5, with the 0.5% dilution being better than the 0.25% dilution. The responses from soil inocula are shown in Table 3 and a response from a pure culture is shown in Figure 3.

To dilute complex constituents further and also to provide a basal medium which would permit the evaluation of radioactive amino acids as substrates, the casamino acids were omitted from M6 resulting in the medium designated M7. Responses from several pure cultures are shown in Table 4. It can be seen that omission of the amino acids was not unduly detrimental to any of the organisms tested and showed a significant advantage for *Saccharomyces cerevisiae*. When tested in chambers using the automatic monitoring system, response from *Escherichia coli* was identical in M6 and M7 and the response from *Staphylococcus epidermidis* was better in M7.

2. Organic-free Basal Media

In an effort to examine the extreme case of simplicity of the basal medium, the response from organisms in soils suspended in aqueous solutions of glucose-C^{14} and sodium formate-C^{14} without any additional constituents was investigated. The assumption was made that soil containing the microorganisms would provide the nutrients necessary for growth and the radioactive substrates would be metabolized with subsequent evolution of $C^{14}O_2$.

Initially, soil extract was used as the solvent for the radioactive glucose (1.6×10^{-3} M) and formate (1×10^{-3} M). The experiments were carried out as follows: each test organism was grown in broth for 24 hours after which 0.5 ml was used to seed 0.5 g of sterilized garden soil. The organisms were incubated in the soil for an additional 24 hours. Two loopsful of

TABLE II -3

RESPONSE FROM DESERT SOILS TO MEDIUM M5 AND DILUTIONS OF YEAST EXTRACT AND PEPTONE

Soil No.	Medium		
	M5	0.5 %*	0.25% *
1	2	1	3
1	3	2	1
68	1	1	2
68	2	2	1
70	2	1	2
70	2	1	2
74	2	1	-
74	2	1	-
76	2	2	1
76	2	1	-

*Concentration of yeast extract and peptone relative to M5.

Same soil numbers represent replicate experiments.

Responses rated as: 1 better than 2 better than 3, and are averages of replicate determinations.

The desert soils were taken from the Mojave Desert and provided by the Space Biology Group, Jet Propulsion Laboratory.

FIGURE II-3

Response of E. Coli To Dilutions Of Yeast Extract And Peptone In M5 Medium

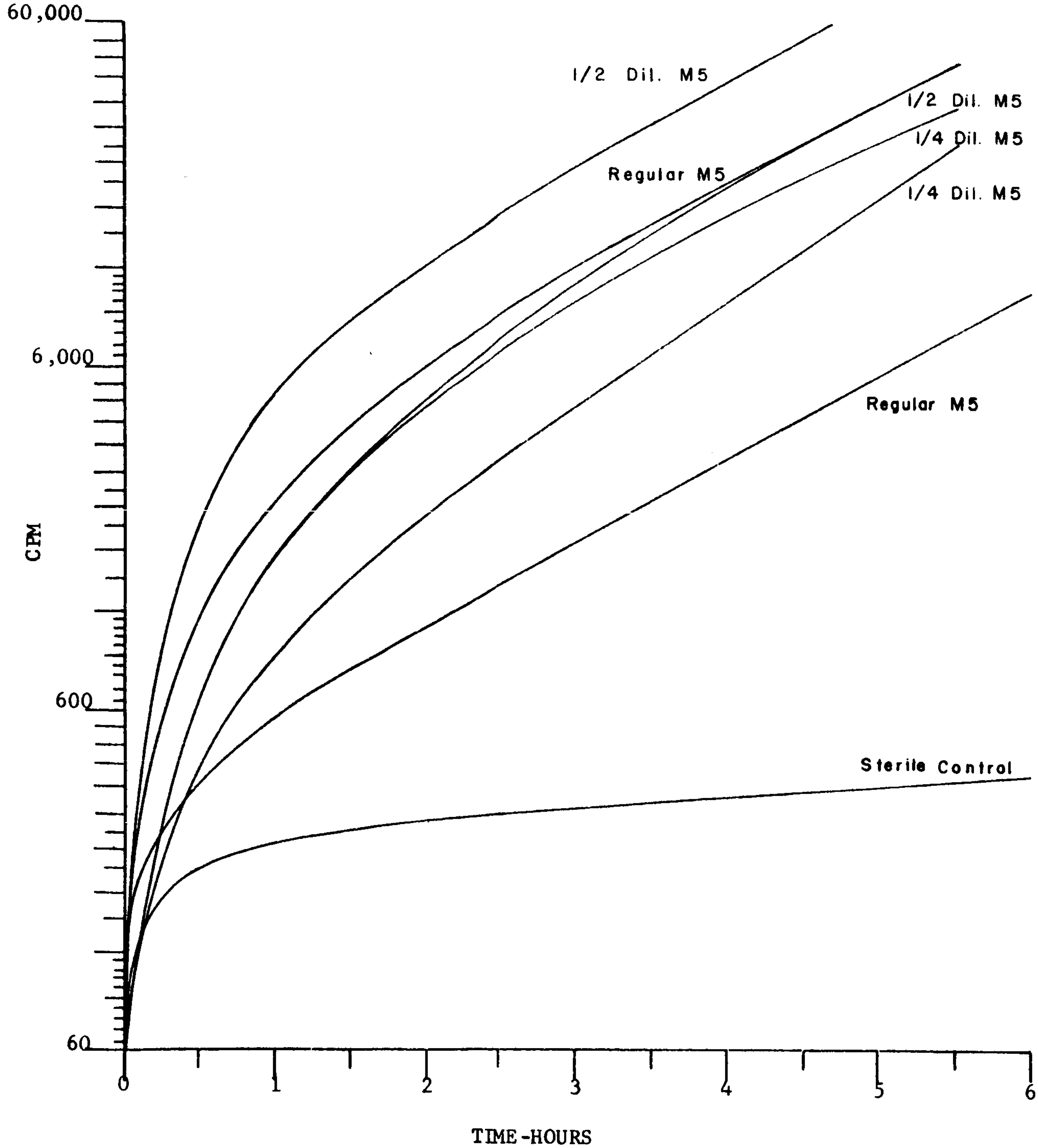


TABLE II-4

RESPONSE OF EQUAL INOCULA OF PURE CULTURES
TO MEDIA M6 and M7

Test Organisms	Response - CPM Above Control M6	M7
Control	47	48
Escherichia coli	8,519	5,582
Micrococcus cinnabareus	601	510
Saccharomyces cerevisiae	353	1,031
Staphylococcus epidermidis	45	67

Total incubation and CO_2 collection time = 3.5 hours in planchets

M6 and M7 - defined in introduction

Values are averages of triplicates

Inocula added as equal volumes from the same broth culture for each organism.

inoculated soil were used to seed each of two sets of planchets. One set contained 0.5 ml of radioactive M5 medium per planchet and the second set contained 0.5 ml of soil extract to which the labeled formate and glucose were added. The concentrations of labeled substrates were the same in both sets. The organisms were incubated at room temperature for three hours and CO_2 collected on pads containing saturated $Ba(OH)_2$ for an additional 30 minutes. Carbon dioxide collections were made again at 5.5 hours and after overnight incubation. When tested in this way, response of the individual test organisms was better in the complex medium (M5) than in the labeled soil extract medium.

It was decided to pursue the investigation of a simple basal medium one step further and to add aqueous solutions of the radioactive substrates directly to various soil inocula. Experiments were carried out in which the response from 100 mg samples of soil, moistened with 0.2 ml of an aqueous solution of formate-C^{14} and glucose-C^{14} was compared to the response from an equal inoculum moistened with 0.2 ml of either M6 or M7 medium containing labeled glucose and formate. Without exception, the simple medium yielded a higher response in a shorter time than either complex medium. A comparison between the aqueous solution and M6 medium is shown in Table 5. When soils were incubated in the chambers and $C^{14}O_2$ was monitored in the automatic unit, the simple medium was again superior to the complex medium in the initial phase. In several determinations, however, the $C^{14}O_2$ evolution from soils in the complex medium exceeded that from the simple medium after 9-12 hours of incubation (Figure 4). It seemed possible that the soil was limiting in some of the inorganic salts that were present in the complex medium. To test this possibility, a comparison was made using soil

TABLE II-5

EVOLUTION OF $C^{14}O_2$ FROM 100 MG SAMPLES OF SOILS INCUBATED IN M6

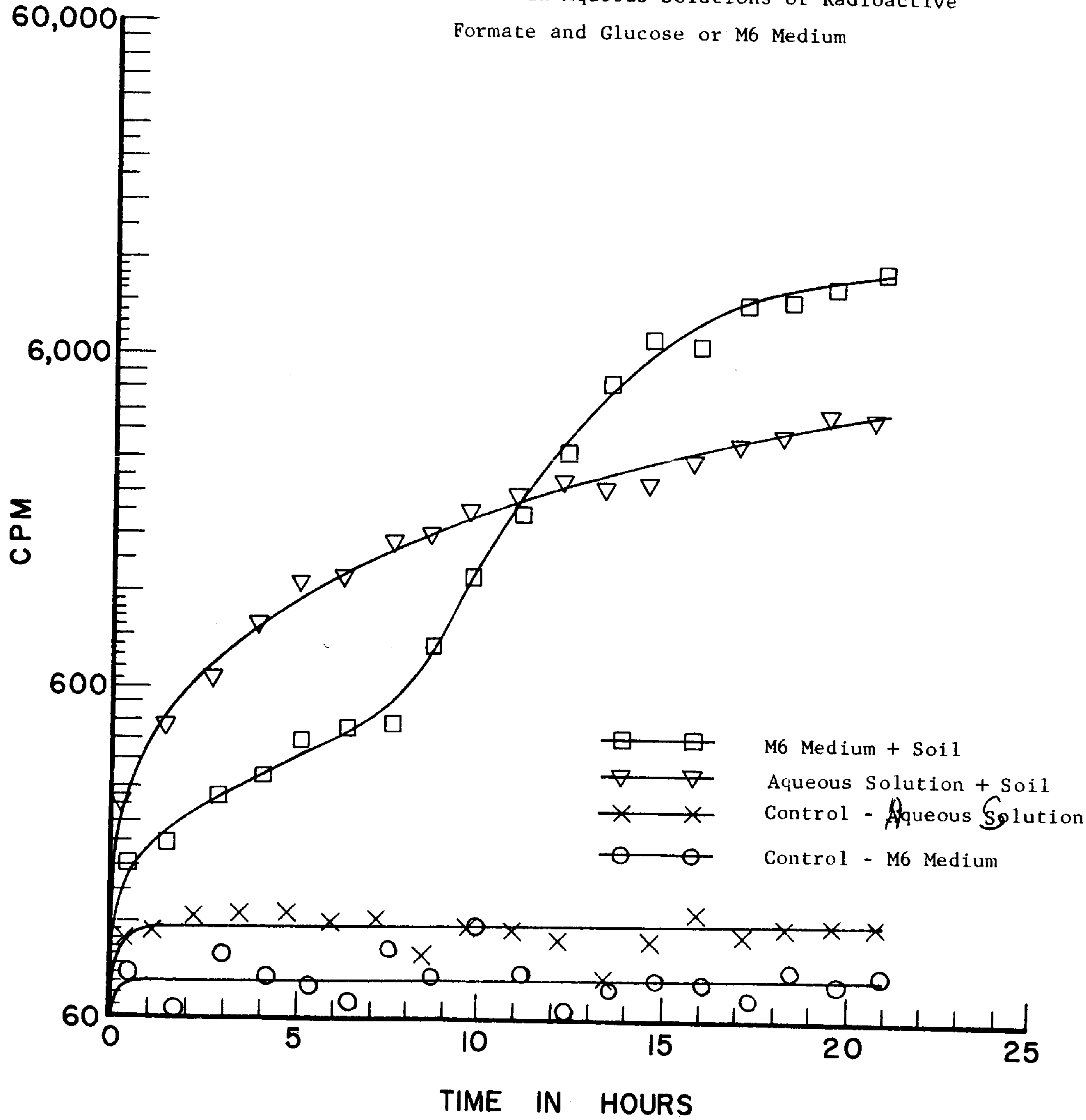
OR IN AQUEOUS SOLUTIONS OF RADIOACTIVE FORMATE AND GLUCOSE

Time Elapsed After Inoculation(Hrs.)	Radioactivity - CPM Above Sterile Control Medium Receiving Soil and Labeled Substrate H_2O	 M6
Experiment A		
0.5	6,747	4,080
1.5	6,888	4,281
Experiment B		
0.5	3,154	1,352
1.5	2,553	1,119
Experiment C		
0.5	1,956	818
1.5	1,848	604
Experiment D		
0.5	1,144	1,040
1.5	1,557	913

Radioactive substrates - glucose (3.2×10^{-3} M) + formate (2×10^{-4} M)
Determination carried out in planchets.

FIGURE II-4

Evolution of $C^{14}O_2$ From 100 Mg Samples of Soils Incubated in Aqueous Solutions of Radioactive Formate and Glucose or M6 Medium



inocula in M7; in an aqueous solution of sodium formate-C^{14} plus glucose-C^{14}; and in M8. As shown above, M8 contains only K_2HPO_4, KNO_3, $MgSO_4$, NaCl, and the radioactive substrates. Results show that M8 and the simple solution of radioactive substrates are always equal to, or better than the complex medium, M7 (Figure 5). Thus far, some advantage has been observed in the salts medium (M8) and a rational approach would be to continue its use. Response from the simple aqueous medium seems to have improved when the concentration of labeled substrates was changed to provide 1 X 10^{-3} M formate and 3.3 X 10^{-4} M glucose. Details of the effect of the concentrations and ratios of formate to glucose are presented below.

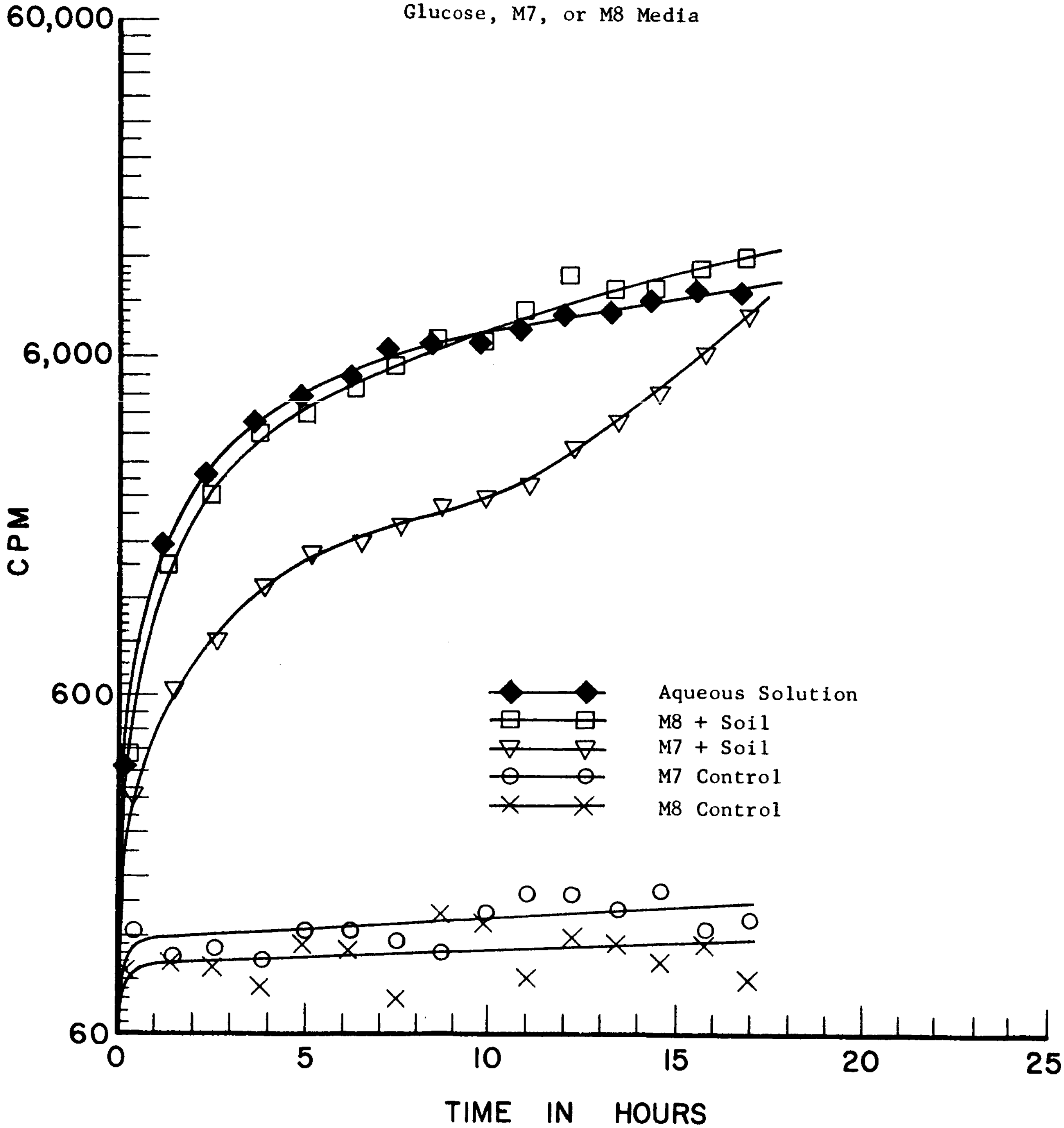
The response to a simple basal medium by organisms in soil is under intensive investigation. Pure cultures of organisms isolated from the test soils will be tested in this medium to determine if the improved response might be due to enhanced metabolism of only several species of organisms which might be common to the many varieties of soil samples tested. If it is determined that the response is general from the many species of organisms present in soils, this aspect of medium development will be an important and highly encouraging result. Pure cultures of test organisms will also be inoculated into the simple medium.

3. Effects of Various Levels of Sodium Formate-C^{14} and Glucose-C^{14}.

The specific activity of the sodium formate-C^{14} used throughout the past year has an average value of 5mc/mM. When used at a level of 1 μc/ml in the medium, the formate results in a weight per volume concentration of 0.002% and a solution which is 2 X 10^{-4} M. The glucose used has an average value of 3.3 mc/mM and at a level of 1 μc/ml, results in a concentration of 0.005% (w/v), and a solution which is 3 X 10^{-4} M.

FIGURE II-5

Evolution of $C^{14}O_2$ From 100Mg Samples of Soils Incubated in Aqueous Solutions of Radioactive Formate Glucose, M7, or M8 Media



Initially, formate was used at 2.5 X 10^{-4} M concentration and glucose at 4 X 10^{-4} M. However, preliminary studies indicated that some advantage in test sensitivity could be gained by increasing the levels of formate to 1 X 10^{-3} M and glucose to 1.6 X 10^{-3} M. This resulted in formate at a weight per volume concentration of 0.010% and glucose at 0.025% and was used for a majority of the determinations throughout the past year.

During the last quarter, more detailed studies have shown that the use of formate at a concentration of 1 X 10^{-3} M and glucose at 3 X 10^{-4} M results in a more rapid response from pure cultures of Escherichia coli and from desert soil inocula. When formate and glucose were compared on the basis of radioactivity in the following ratios, and actual amounts, for each ml (µc formate to µc glucose): 1/1, 1/5, 1/10, 5/5, and 5/1, using the 1/1 ratio as the standard, the only definite increase in response resulted from the 5/1 ratio, indicating a metabolic effect other than a mere increase in concentration. Thus, the relative importance of formate is revealed.

4. New Substrates

a. Carbon-14 Compounds

In an effort to gain maximum advantage from the medium, radioactive substrates in addition to formate and glucose were incorporated into the basal M8 medium. The new substrates tested to date are: DL-aspartic-4-C^{14} acid, specific activity - 2.0 mc/mM; DL-glutamic-1-C^{14} acid, specific activity - 3.0 mc/mM; DL-sodium lactate-1-C^{14}, specific activity 5.0 mc/mM.

Since the radioactive ratio of 5 µc formate to 1 µc glucose appeared to offer an advantage over other combinations tested, it was decided to maintain those compounds in that ratio, and also to maintain the total concentration of labeled substrates at a constant molar level. Because the specific activity of the formate is 5 mc/mM and the specific activity of the glucose is 3 mc/mM, a 5 μc/1 μc ratio yields a 3/1 ratio on a molar basis. Each ml of basal medium then contains 1 μM of sodium formate-C^{14} and 0.33 μM of glucose-C^{14}, a total of 1.33 μM. This level of formate and glucose was used as the standard to which other combinations or single compounds were compared. When the new substrates were added, the formate and glucose concentrations were reduced to 0.66 μM/ml and 0.22 μM/ml to maintain the 3/1 ratio. Each new substrate was added at a level of 0.44 μM/ml, to maintain the total 1.33 μM/ml. In addition to the above combinations, each new substrate was tested on an individual basis at a level of 1.33 μM/ml. With the molarity constant, the μc/ml of each labeled substrate, or combination of labeled substrates varied, being dependent, of course, upon the specific activities of the radioactive substrates incorporated into the medium. The range of activity was from 2.6 μc/ml when aspartic acid was used alone, to 6.7 μc/ml when the lactic acid was used alone.

The automated $C^{14}O_2$ monitoring unit was used for all of the studies. Two-tenths ml of the desired labeled M8 medium was inoculated with 100 mg of soil, and the resulting $C^{14}O_2$ evolution monitored and automatically recorded.

Responses from a soil inoculum with the aspartic-C^{14} acid and with the glutamic-C^{14} acid in combination with formate-C^{14} and glucose-C^{14} were not any higher than they were with the formate and glucose combined. However, aspartic and glutamic were each used alone to a small extent, indicating their availability to the soil organisms. Their utilization under the test conditions was so slight that they will not be incorporated into the medium at present.

However, the situation with the sodium lactate-1-C^{14} is different. Responses from soil inocula when lactate was added to the formate and glucose were equal to, or higher than, the formate-glucose combination. At the same time, response from lactate alone was nearly as good as the glucose and formate combined or the glucose-formate-lactate combination (Figure 6). Because of the specific activity, the radioactivity of the lactate in the medium was 6.7 μc/ml and higher than the aspartic (2.6 μc/ml) and glutamic (4.0 μc/ml) alone. This might contribute to the degree of response but does not alter the fact that, as incorporated into the medium, the lactate was readily utilized by the soil organisms. Attempts will be made to establish the range of organisms which produce CO_2 from lactate. If indicated, lactate will be incorporated into the basal medium.

b. Tritium

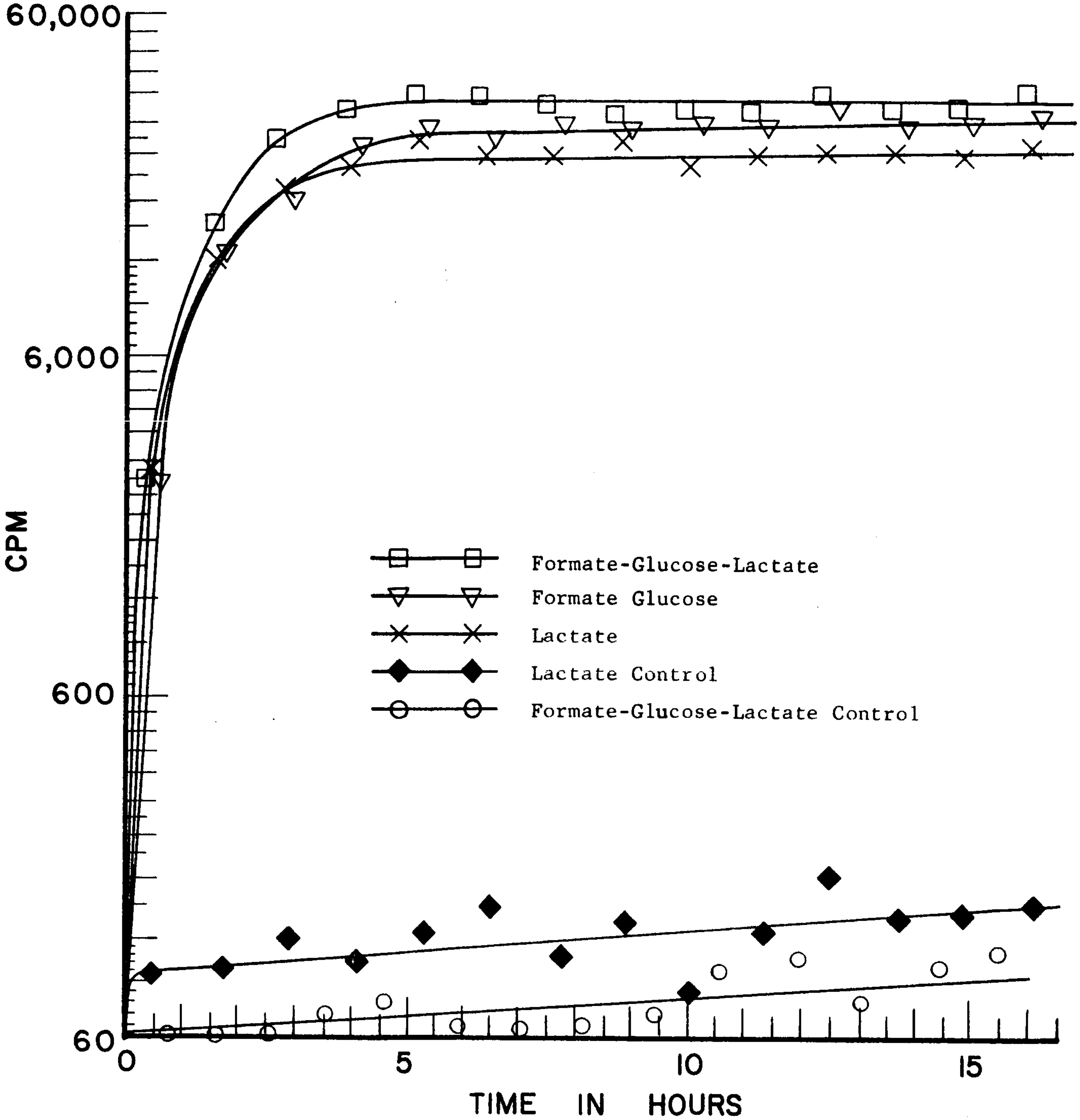
A study was made to determine the possibility of using tritium labeled compounds in the medium. It was concluded that compounds labeled by the Wilzbach Technique, as originally proposed, would exchange hydrogen too readily for use. Other methods of labeling might be satisfactory but as yet no practicable approach to using tritium substrates and detecting evolved tritiated gases has been developed.

C. STERILE CONTROLS

As previously reported, the radioactive substrates presently used yield some $C^{14}O_2$ even when sterile. The laboratory procedure for sterilizing media containing sodium formate-C^{14} and glucose-C^{14} by autoclaving at 15 psi for 15-20 minutes and storing it in a refrigerator until needed is

FIGURE II-6

Evolution of $C^{14}O_2$ From 100 Mg. Samples of Soil Incubated in M8 Medium with Formate-Glucose, Formate-Glucose-Lactate, or Lactate Alone as Radioactive Substrates.



quite satisfactory and results in low sterile control levels. However, several observations of importance have been made and require consideration. First, if the freshly sterilized media are assayed for $C^{14}O_2$ evolution immediately after autoclaving, the nonmetabolic evolution begins at a relatively high level and drops to just above background upon standing in flasks plugged with cotton. The rate of decrease appears to be a function of surface/volume. Secondly, when the media are sealed in ampules and heated at 135°C for 26 hours in dry heat, the nonmetabolic activity obtained from the media when removed from the ampules is higher than when autoclaved. The rate of evolution of nonmetabolic $C^{14}O_2$ decreases when the media stand. Thirdly, the formate appears to contribute more to the nonmetabolic $C^{14}O_2$ level than the glucose. Fourthly, the nonmetabolic level is higher when the radioactive substrates are heated in the complex media than when they are heated in water. The actual levels of radioactivity obtained vary with the concentration of labeled substrate and have at times been in excess of 500 cpm when assayed immediately after opening. In several instances, media sealed in ampules and heated in the instrument for field testing have been even higher prior to flushing.

Two approaches are being made in an effort to maintain the nonmetabolic activity at a minimum. One approach is to utilize means of flushing the broth as soon as it is released from the ampule. Most of this study is being performed at AMF and is reported in the section on instrumentation.

The second approach seeks to determine the conditions responsible for the nonmetabolic $C^{14}O_2$. An effort is being made to establish the extent to which radioactive decay contributes to the evolution of $C^{14}O_2$ from the sterile medium and to study the influence of other factors such as heat, oxygen, and chemical impurities on the decomposition of the labeled substrates.

Since sodium formate has been observed to be more of a problem than glucose, initial efforts have been concentrated on it. The observations mentioned above have resulted from laboratory studies using sodium formate-C^{14}. Variations in the absolute amount of radioactive CO_2 detected occur as a consequence of the manner in which the formate is handled and in which the $C^{14}O_2$ is collected and assayed. For that reason, only general observations appear warranted at the present time. As stated previously, heating does result in increased nonmetabolic radioactivity. However, unheated, freshly prepared sodium formate in distilled, deionized water stored in sealed ampules also yields $C^{14}O_2$ when the ampules are broken.

The radiation chemistry of aqueous solutions of formic acid has been investigated rather extensively and the rate of decomposition resulting from C^{14} decay can be ascertained. However, the decomposition rate and amount of this degradation which results in CO_2 varies with experimental conditions. The degree of decomposition of crystalline sodium formate-C^{14} (sp.act. 4.53 mc/mM) would be about 0.012% daily if one assigned the rather high G value of 10. Radiation chemical yields are expressed as "G", which represents the number of molecules of material formed or destroyed for each 100 ev deposited in the substance. If it is further assumed that CO_2 is the only product of decomposition, the total radioactivity produced every day would be equal to 4×10^3 dpm. This value represents the maximum nonmetabolic activity that could result from internal radiation. Work by Hart (J.A.C.S. 76: 4312,1954) on aqueous solutions of sodium formate at the same concentrations used in Gulliver, but at pH values of around 3.0 shows that the actual G value for CO_2 formation varies between 2 and 3 depending upon the amount of oxygen present. The internal radiation dose in Gulliver is less than the radiation doses used by Hart. On this basis, the amount of decay in Gulliver should be less than 25 per cent of the value calculated above as a maximum. The actual amount detected will depend upon detector efficiency. However, even less CO_2 would evolve since the pH of the medium in Gulliver will be about 7.0, and it has been shown that less CO_2 results from decomposition as the pH level increases (Garrison, W.M., B.M. Weeks and S. Cole, Nature 193:1291,1962). In fact, Hardwick and Guentner (J.Phys.Chem.63:896,1959) have shown that at pH 7.5 and above, there is no detectable CO_2 resulting from decomposition of sodium formate. The products identified by them are hydrogen, sodium oxalate plus small amounts of formaldehyde and glyoxalic acid. The pH effect has been observed by other investigators, and several reaction mechanisms have been postulated (Garrison *et al*).

The problem is to select the most effective control measures to minimize CO_2 formation. This might be accomplished by packaging the radioactive compounds as solids in a thin layer or by utilizing "scavenger" solvents. Possibly the most practicable means of controlling the formation of nonmetabolic $C^{14}O_2$ is to utilize a pH level of 7.5 or above in the medium and reduce it before it contacts the soil sample. Such a system might or might not eliminate the need for terminal offgassing of the medium on arriving at its destination.

D. ANTIMETABOLITES

An antimetabolite in a control chamber would add immensely to the significance of the test. However, in many respects, the search for a "universal antimetabolite" is as difficult as the development of a "universal medium". The antimetabolite for Gulliver must be: active against a broad spectrum of microorganisms in a soil-type environment; heat stable; unreactive with the radioactive substrates to preclude production of nonmetabolic $C^{14}O_2$;

and must be readily diffusible throughout the test chamber.

Antimetabolite studies have been carried out using the planchet method in order to permit an extensive screening program at a reasonably low cost in time and isotopes. Planchets were seeded in triplicate and compared to unseeded controls. Each test set contained medium alone or medium plus inhibitor. The planchets were incubated for two hours after which CO_2 was collected for 15 minutes. The cultures were incubated for an additional two hours and CO_2 again collected for 15 minutes. The CO_2 collection pads were dried and the radioactivity assayed in a Nuclear-Chicago D-47 end window counter.

A number of chemicals and commercial disinfectants were screened in this manner. They included acetic acid; barium hydroxide; calcium hypochlorite; Airkem A-3 (n-alkyl dimethyl benzyl ammonium chloride, Na_2CO_3, tetrasodium ethylene diamine tetracetate, nonylphenoxy polyoxyethylene, ethanol, and essential oils); Lysol (soap, orthohydroxy-diphenyl, alcohol, pine oil, propylene glycol and glycerol); Amphyl (potassium ricinoleate, o-phenylphenol, p-tertiary amylphenol, alcohol, propylene glycol, and glycerol); and Bard-Parker Germicide (isopropanol, methanol, formaldehyde, and hexachlorophene). The final concentration of each was: acetic acid - 10%; barium hydroxide - 10%; calcium hypochlorite - 2%; Airkem A-3 - 10%; lysol - 2%; Amphyl - 2%; and Bard-Parker Germicide - 10%. Initially, the screening was carried out without first heating the inhibitors to the prescribed sterilization temperatures for the space craft. Subsequently, $Ba(OH)_2$, Amphyl, Bard-Parker, Lysol, and Airkem A-3 were tested after being heated to 135°C for 26 hours and cooled to room temperature. Results from pure cultures are summarized in Table 6.

TABLE II-6

EFFECTS OF INHIBITORS ON $C^{14}O_2$ EVOLUTION FROM PURE CULTURES

Organism	Inhibitor						
	Calcium Hypochlorite	Barium Hydroxide	Acetic Acid	Lysol	Amphyl	A-3	Bard-Parker
Escherichia coli	+	-		+	+	+	+
Pseudomonas delphinii							+
Pseudomonas maculicola	-			+	+		+
Pseudomonas fluorescens							+
Bacillus subtilis	+	-		-	±	+	+
Bacillus subtilis spores					±		-
Saccharomyces cerevisiae	-	-		±	±	+	+
Staphylococcus epidermidis							+
Azotobacter agilis		+	+	+	+		+
Azotobacter indicus					-		+
Streptomyces fradiae		+	+	+	+		+
Streptomyces bobiliae							±
Xanthomonas campestris		+	±	±	+		+

+ = inhibition
- = no inhibition

TABLE II-6 (cont.)

EFFECTS OF INHIBITORS ON $C^{14}O_2$ EVOLUTION FROM PURE CULTURES

Organism	Inhibitor						
	Calcium Hypochlorite	Barium Hydroxide	Acetic Acid	Lysol	Amphyl	A-3	Bard-Parker
Xanthomonas beticola					+		+
Chlorella pyrenoidosa		-		+	+	-	+
Micrococcus cinnabareus							+
Rhizobium leguminosarium		-		+	+	+	+
Bacterium bibulum							+
Mycobacterium phlei		-		+	+	-	±
Arthrobacter simplex							±
Rhodospirillum rubrum							+
Clostridium Sporogenes				+			+

+ = inhibition
- = no inhibition

Antimetabolite tests against soil inocula have only begun, but the response is encouraging. Duplicate determinations from 100 mg garden soil inocula in planchets resulted in an average of 7,132 cpm from one series and 7,762 cpm from a second series after 30 minutes incubation without inhibitor. After the initial 30 minute incubation, Bard-Parker Germicide was added to the 7,762 cpm set and both sets were reincubated for an additional 30 minutes. The first set then read 7,269 cpm and the activity of the inhibited set decreased from 7,762 cpm to 766 cpm. After an additional hour of incubation, the uninhibited set produced an additional 7,142 cpm against the inhibited set's 442 cpm. Carbon dioxide was collected for 15 minutes for each determination. In summary, a soil inoculum which was permitted to respond uninhibited for 30 minutes prior to the addition of antimetabolite yielded 7,762 cpm, but 30 minutes after Bard-Parker Germicide was added, only yielded 766 cpm. An uninhibited control continued at a level of 7,269 cpm. This shows that Bard-Parker can inhibit organisms in soil even after a reasonably high respiration rate has been established.

Bard-Parker Germicide was also added during field testing with apparent effectiveness (Figure 16).

Several conclusions can be drawn from the data obtained to date. Calcium hypochlorite caused excessive instability in the uninoculated medium and was not particularly effective as an antimetabolite at the concentrations used. Barium hydroxide was not adequate against the more vigorously growing cultures. Acetic acid resulted in high control values. In general, the commercial disinfectants had some inhibitory effect, but results were somewhat inconsistent and sterile control values often high. Of these, the Bard-Parker Germicide has been the most promising, even after being heated, but still has not been completely satisfactory. Its effect on a garden soil inoculum is encouraging, but requires more extensive investigation.

In order to eliminate errors in interpreting the inhibitory effects of the Bard-Parker Germicide, the pH values were determined. The pH of the disinfectant was unchanged after heating, and its addition did not change the pH of the growth medium appreciably. Heated Bard-Parker was also tested in a disc assay against Escherichia coli, Bacillus subtilis, and Mycobacterium phlei and was effective against all of them.

Inhibitor studies will continue in an effort to find the most reliable antimetabolite. Ethylene oxide and other inhibitors still to be selected will be evaluated; including the possible use of heat.

E. EFFECT OF TEMPERATURE ON METABOLIC $C^{14}O_2$ EVOLUTION

To determine the effect of temperature on CO_2 evolution from organisms in pure cultures and soils, several types of determinations were made. In one investigation, 75 mg soil samples were incubated at 20°C and duplicate samples were incubated at 30°C. Carbon dioxide was collected on solid, $Ba(OH)_2$- coated, aluminized Mylar and then assayed with a Nuclear-Chicago Labitron. The activity at 30°C was approximately 2.5 times the activity at 20°C, a value suggesting the standard Q_{10} for chemical reactions.

In a different type of determination, experiments were performed in which inocula of the test organisms were incubated at room temperature and equal inocula were incubated at low temperatures (4-8°C). Two types of inocula were used. In one case, pure cultures of organisms were grown in broth for 24 hours, 0.5 ml transferred to 0.5 mg of sterilized soil for an additional 24 hours and then two loopsful introduced into 0.5 ml of M5 medium. In another test, 10 mg samples of soils which were collected and handled aseptically were used for inocula. Both types of inocula were incubated at room temperature and at reduced temperature in planchets. Carbon dioxide was collected in pads containing saturated $Ba(OH)_2$, and radioactivity was monitored in an internal flow counter. Responses generally diminished at reduced temperatures (Tables 7 and 8). However, compared to sterile controls, the response was measurable, with the response from Pseudomonas maculicola and the response from soils being quite high. Although Pseudomonas delphinii, Pseudomonas maculicola, and Photobacterium phosphoreum may be classed as psychrophiles, their growth rate is reduced at low temperatures, a fact which is reflected in the reduced rate of metabolism detected radioisotopically.

Two points should be noted. First, metabolism can be detected from organisms incubated at temperatures below their optimum growth temperatures. These organisms were probably in lag or resting states, illustrating the capability of the radioisotopic method for detecting life without the necessity of reproduction. Second, Martian microorganisms presumably would be capable of reproducing at lower temperatures thereby facilitating detection.

Other cultures of psychrophiles will be obtained in the near future, and will be utilized in the screening program.

F. PHOTOSYNTHESIS

One of the most significant experiments to be considered for Mars is one which would establish the presence of photosynthesizing organisms. Because Gulliver is basically a respirometer, it is conceivable that it can be modified in a manner which would permit its use for radiorespirometric measurements of metabolic processes peculiar to photosynthesis. Toward this

TABLE II-7

EFFECT OF TEMPERATURE ON $C^{14}O_2$ EVOLUTION BY

VARIOUS TEST ORGANISMS

ORGANISMS	Radioactivity - CPM Above Control					
	25°C			4-8°C		
	3.5 Hrs.	5.5 Hrs.	24 Hrs.	3.5 Hrs.	5.5 Hrs.	24 Hrs.
Rhodopseudomonas capsulata	365	167	2,468	13	215	52
Pseudomonas delphinii	971	1,697	383	212	124	112
Pseudomonas maculicola	16,266	4,557	211	1,826	1,215	536
Photobacterium phosphoreum	8	2,423	2,220	0	60	1,800
Escherichia coli	1,586	7,436	-	60	72	-
Bacillus subtilis spore suspension	11,784	32,367	722	291	1,356	887

Medium -M5 with C^{14} sodium formate (1.0×10^{-3} M) plus C^{14} glucose (1.6×10^{-3} M)

TABLE II-8

EFFECT OF TEMPERATURE ON $C^{14}O_2$ EVOLUTION
FROM ORGANISMS IN SOIL SAMPLES

SAMPLE	Radioactivity - CPM Above Control									
	25°C					8°C				
	30 min.	2 hrs	3 hrs	4 hrs	24 hrs	30 min	2 hrs	3 hrs	4 hrs	24 hrs
Field -approx 10 mg	3,802	734	-	4,886	8,271	476	355	-	513	450
Control-sterile medium	25	25	-	26	26	25	25	-	26	27
Field-approx. 10 mg	6,914	7,975	5,341	16,077	15,966	1,453	1,878	1,343	1,615	2,206
Control-sterile medium	73	78	82	73	67	27	25	20	28	27

Medium - M5 with C^{14} sodium formate (1.0 X 10^{-3} $\underline{M}$) plus C^{14} glucose (1.6 X 10^{-3} $\underline{M}$)

goal, work was begun to investigate the feasibility of using Gulliver as a photosynthetic experiment.

Two approaches which might be utilized are: (1) to supply $C^{14}O_2$ and determine the amount assimilated or fixed by photosynthesizing organisms or, (2) to determine the effects of light on the respiration and net $C^{14}O_2$ evolved from labeled substrates by photosynthesizing organisms. It seemed likely that photosynthetic organisms which could assimilate labeled substrates would utilize some of the $C^{14}O_2$ produced by that assimilation in photosynthesis. Thus, a difference in net $C^{14}O_2$ evolved from a culture would result, depending on whether the culture were maintained in light or dark. Because the second approach more nearly conforms to the present capability of Gulliver it was selected as the one to investigate first.

Chambers shown beneath the Geiger tubes in Figure 1 were modified slightly to make them functional as light chambers. This was accomplished by replacing one side with a sliding panel, inserting a small pyrex test tube through the top of the chamber, and making a gas tight seal around the top of the tube. Radioactive medium can be placed in the tube, seeded with organisms, and exposed to light or maintained in the dark through the use of the panel. The $C^{14}O_2$ evolved can be collected and monitored exactly as described with the automatic unit. Because the side panel is removable, cultures can be incubated initially in the dark and changed to light, or vice versa.

Another method of collecting the evolved $C^{14}O_2$ has also been used. In this method an aluminum planchet containing a pad saturated with $Ba(OH)_2$ is used in place of the $Ba(OH)_2$-coated Mylar. The chambers are modified only in that a removable panel is placed over the open top. The introduction of medium and organisms into the test tube is the same as described above. The top panel is moved into place thereby sealing the inner chamber, and the culture is allowed to incubate for a period of time, usually ten minutes. At the end of this time a planchet is inverted over the chamber, the panel is moved to the open position, and gas is collected, usually for five minutes. The cycle is repeated throughout the period of the experiment.

A number of experiments have been carried out in an effort to establish the best conditions for detecting $C^{14}O_2$ from a culture of Chlorella pyrenoidosa which has not been purified but contains a tenacious single contaminant which appears to be a Gram negative rod.

The basic procedure is as follows: a radioactive medium is introduced into the sterile tubes in the chambers and, except for sterile controls, the medium is seeded with a culture of Chlorella. One set of chambers is exposed to light, the second set has the side panels in place and is maintained as the dark phase of the experiment. The cultures are allowed to incubate for one hour at room temperature (22°C) after which a $Ba(OH)_2$-coated Mylar CO_2 collector is placed over the baffled, open end of the chamber. The $C^{14}O_2$ is assayed by Geiger Müller tubes via the automatic monitoring unit. In most of the determinations, the closed panels were moved to the open position, and the open panels were moved to the closed position during the course of the experiment, thereby changing the initial light phase chambers to dark phase, and vice versa.

This initial procedure has been modified only to the extent that light and dark control chambers were added. These control chambers are prepared in the same manner as the experimental chambers. The light control was maintained as a light chamber throughout the experiment and the dark control chamber remained in the dark during this period.

A number of media variations have been tried. Initially, liquid M6 containing formate-C^{14} and glucose-C^{14} in a one-to-five radioactivity ratio was used. A response in the form of evolved $C^{14}O_2$ was always obtained but the influence of the light was not always apparent. A change was made to an inorganic medium to which urea was added to supply nitrogen to the culture. Urea was selected because it discourages bacterial contaminants, growth of Chlorella is faster than when nitrate is used, the pH of the culture remains relatively constant, and a large amount of the compound can be introduced into the culture without depressing growth. The medium is composed of the following nutrients made up to one liter with distilled water:

	Urea	0.50 g
	$MgSO_4 \cdot 7H_2O$	0.25 g
	KH_2PO_4	0.25 g
Sequestrene	Fe	30.0 mg
"	Mn	3.0 mg
"	Cu	1.0 mg
"	Zn	1.0 mg
"	Co	1.0 mg

Either D-glucose-C^{14} or DL-sodium lactate-1-C^{14} was used in the medium as the sole radioactive substrate. In this medium containing either glucose or lactate, the response from dark-phase cells has been initially higher than from light-phase cells. Upon changing the dark cells to light phase, the response decreased. Conversely, the light cells showed a marked increase in response when changed to dark phase.

One further change in experimental procedure was made. This was the addition of 1.0 or 1.5% agar and the seeding of the surface of the radioactive medium rather than inoculation of a liquid medium. The type of response, obtained using this procedure and D-glucose-C^{14} as substrate, is shown in Figure 7. The automatic unit was used to monitor this experiment. In a procedure using the same type of medium and substrate, but in which $C^{14}O_2$ was collected by the planchet method, the type of response shown in Figure 8 was obtained. A response from a planchet study in which urea medium was supplemented with DL-sodium lactate-1-C^{14} is shown in Figure 9. In all of the experiments described, the effect of light is clear and seems to be most pronounced early in the experimental periods.

An experiment was conducted with an isolate of the bacterial contaminant observed in the algal cultures. The bacterium was introduced into chambers containing urea medium and D-glucose-C^{14}. The previously described light-dark procedure was utilized and $C^{14}O_2$ was collected in planchets. The result of the experiment is shown in Figure 10. There is no apparent response to light change by the bacterium, thereby indicating that when glucose is used, the response indicated in Figures 7, 8, and 9, is a function of the effect of light on the respiration of photosynthesizing cells and not a function of light on the bacteria.

In a study in which urea-C^{14} was used as the sole nitrogen and carbon source, no response to light change was observed.

Glucose may not be the only, or even the most appropriate, substrate with which to demonstrate the effect of light on the respiration of photosynthesizing cells. In fact, it may be the least effective because the algae may prefer to metabolize it even in the light as a source of energy rather

FIGURE II-7

Response of Chlorella Pyrenoidosa to Light Change

When Cultured in Urea Medium Containing D-Glucose-C^{14}

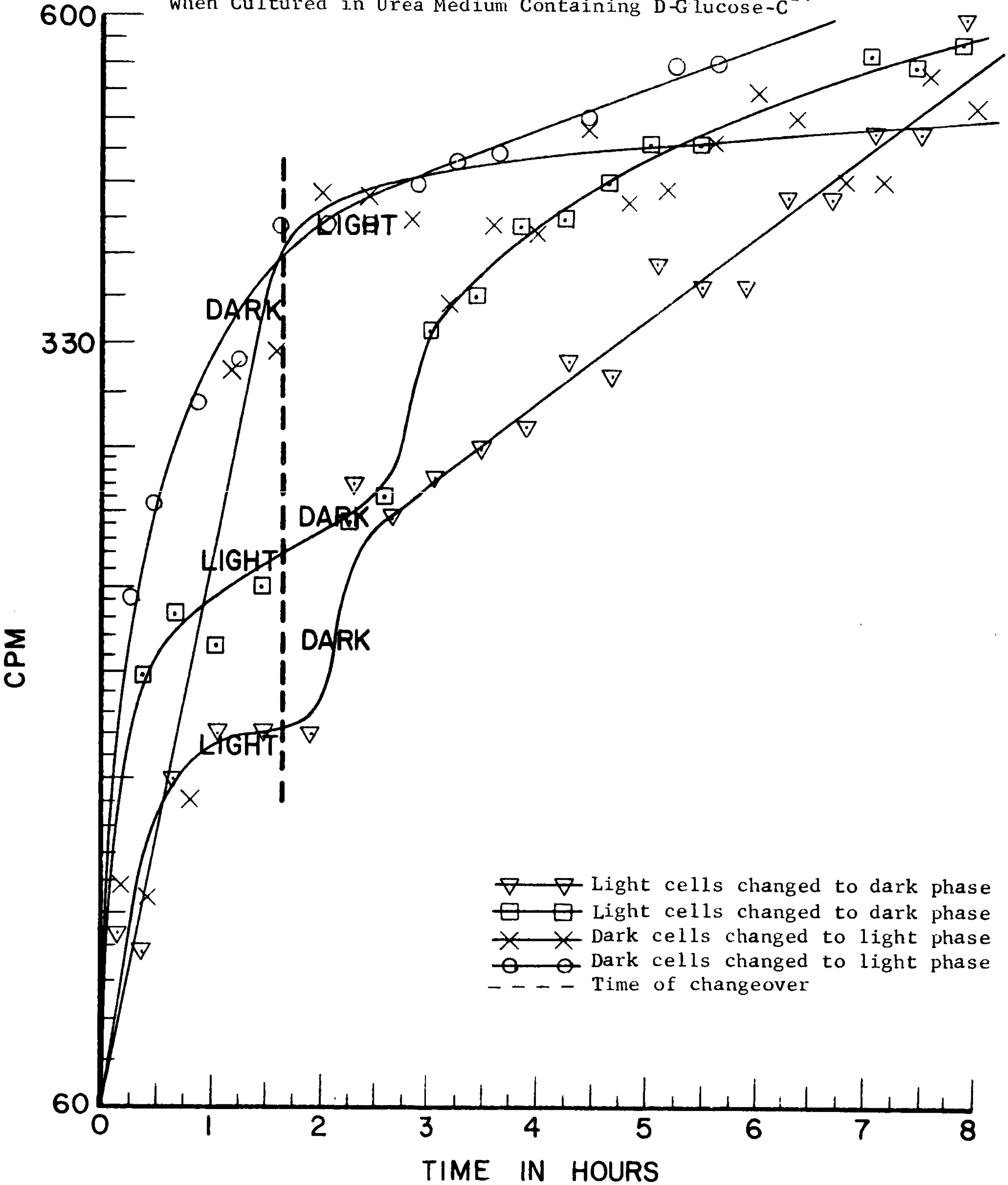


FIGURE II-8

Response of Chlorella Pyrenoidosa to Light Change

When Cultured in Urea Medium Containing D-Glucose-C^{14}

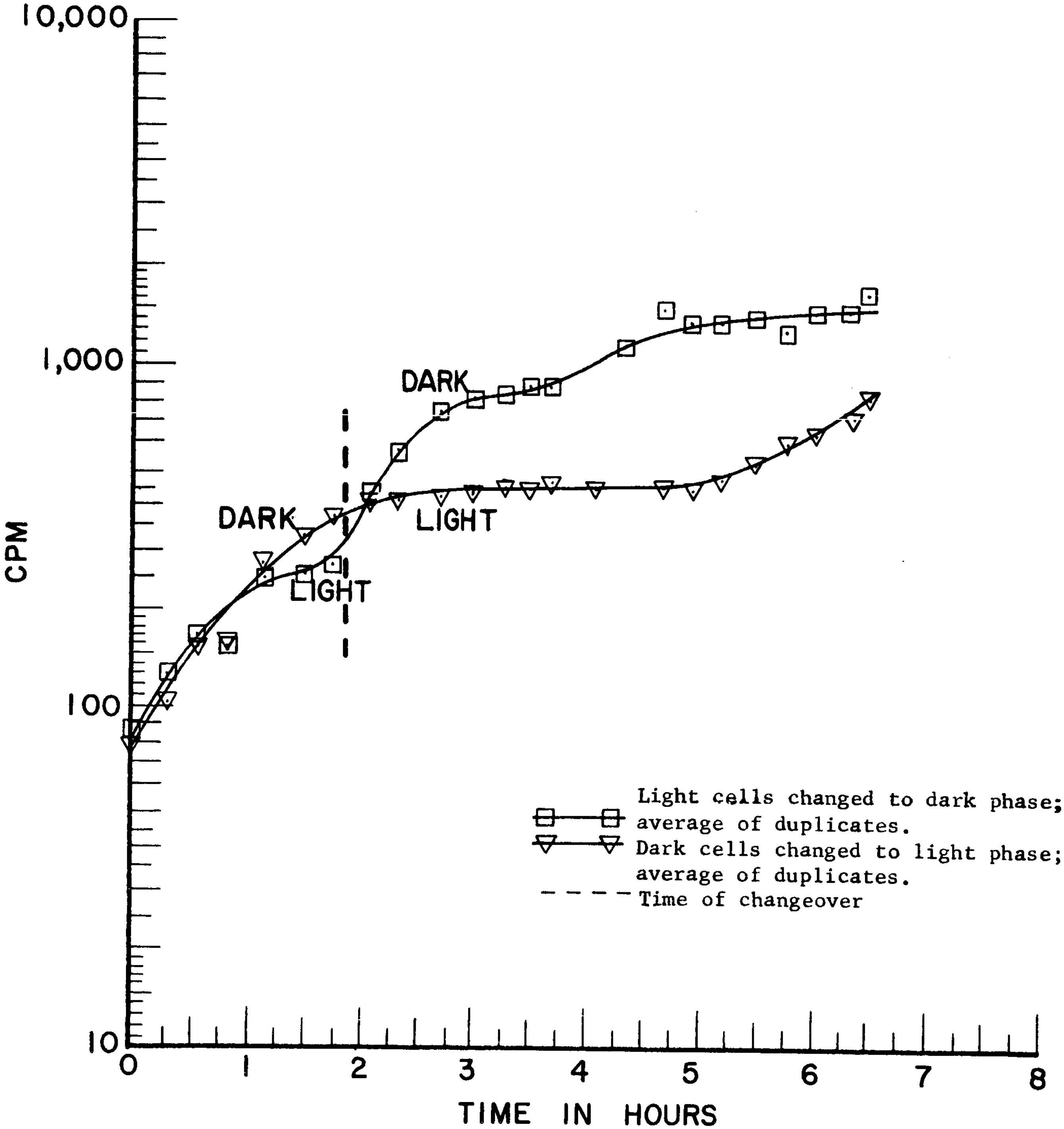


FIGURE II-9

Responses of Chlorella Pyrenoidosa to Light Change When Cultured in Urea Medium Containing DL-Sodium Lactate-1-C^{14}

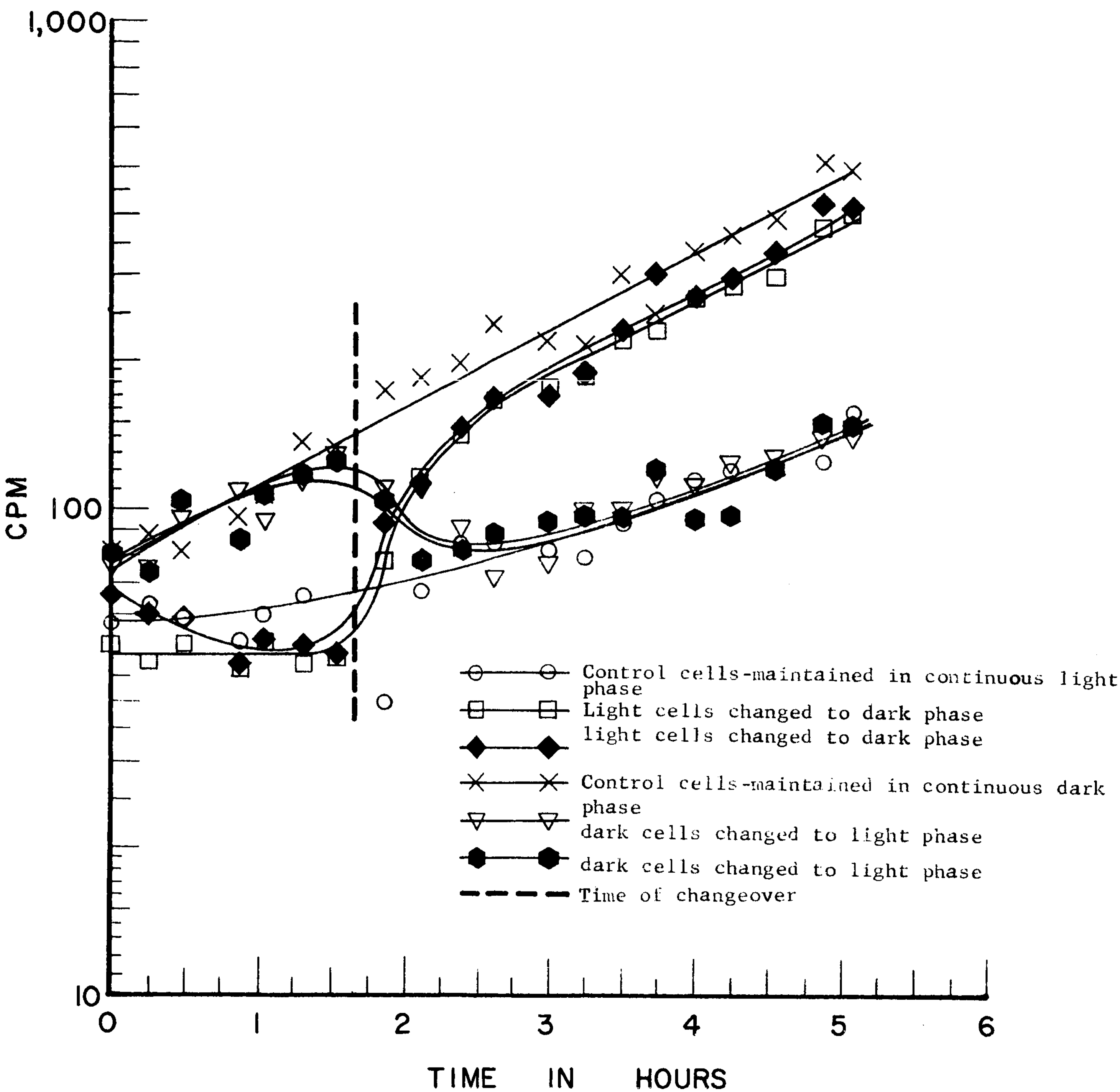
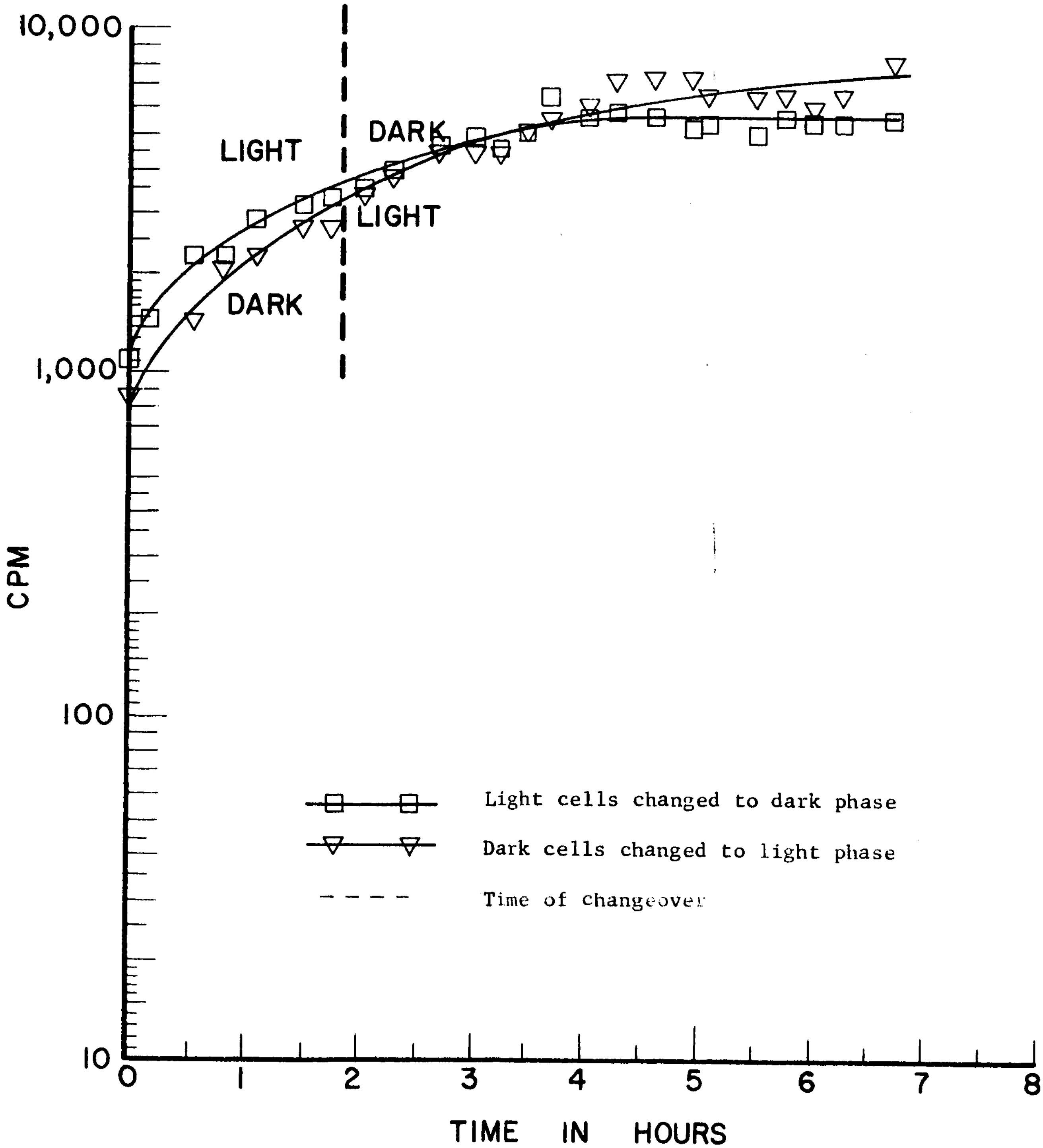


FIGURE II-10

Response of Bacterial Contaminant to Light Change When Cultured in Urea Medium Containing D-Glucose-C^{14}



than other energy sources synthesized photosynthetically. Results of the limited work done with DL-sodium lactate-1-C^{14} indicate that it may be a better substrate than glucose. Other substrates and basal media will also be examined.

The results of the investigation thus far indicate that there is a very marked response to light-dark change by Chlorella pyrenoidosa, with a resultant increase or decrease in evolved $C^{14}O_2$ from some labeled substrates. The data further indicate that the detection system incorporated in Gulliver is capable of determining the very rapid change in evolved CO_2 that occurs as a result of light-dark changes. With this evidence, there is a high degree of probability that Gulliver can be used for carrying out a photosynthetic experiment.

G. GUIDELINES FOR REDESIGN OF INSTRUMENT

A modification in instrument design has been made which permits the unit to function independently of the position of the instrument capsule after it comes to rest on the surface of the planet. This modification is described in the section on Instrumentation. However, in order to assure an adequate response, a number of biological determinations had to be made before modifying the instrument. The biological guidelines used for redesign of the instrument are discussed below:

1. Use of Solid Support for Media

The development of an instrument requiring no attitude control appeared to be more feasible if the requirement for transferring broth from chamber to chamber could be eliminated. Toward this end, a number of studies were conducted utilizing semi-solid and solid media as well as various supporting materials which would give liquid media the same physical characteristics as solid media.

In one approach, small pieces of various synthetic and natural sponges, wool, plush, chenille, or cotton fringe were used as supports for liquid media. The solid materials were placed inside the culture chambers used in the automatic monitoring unit and saturated with M5 medium containing labeled substrates. Soils or pure cultures of cells were placed on the top surface of the material, CO_2 collectors were placed over the chambers and $C^{14}O_2$ evolution was monitored. Responses from pure cultures and desert soil inocula were more rapid and quantitatively superior on solid media when compared to equal inocula in liquid media (Figure 11). However, data indicate that rather than introduce any kind of material into the chamber, (a procedure which would require transferring the soil), it is more practicable and beneficial to use the retrieval line itself as the solid support for the medium. This is accomplished by reeling the retrieval line into the culture chamber and introducing medium through the spindle, thereby saturating the line from the center to the outside. In this way, all of the soil sample is retained and the retrieval line forms a solid support. The quantity of broth added is just sufficient to saturate the string, thereby preventing splashing in the chamber.

The effectiveness of this approach was apparent from a number of determinations in which the retrieval line was compared to various supporting substrates. Comparisons were made by using 46 ft. lengths of sterile retrieval lines to collect soil samples from Rock Creek Park. Two lines were kept on reels and saturated with radioactive medium and two were aseptically scraped to cause the soil to fall onto the solid material. The type of response obtained is shown in Figure 12. These data established

Response From Desert Soil Tested With Liquid And Solid Media

25 mg Soil From Thermal, Calif. (# 1 - 1/10 in)

Figure II-11

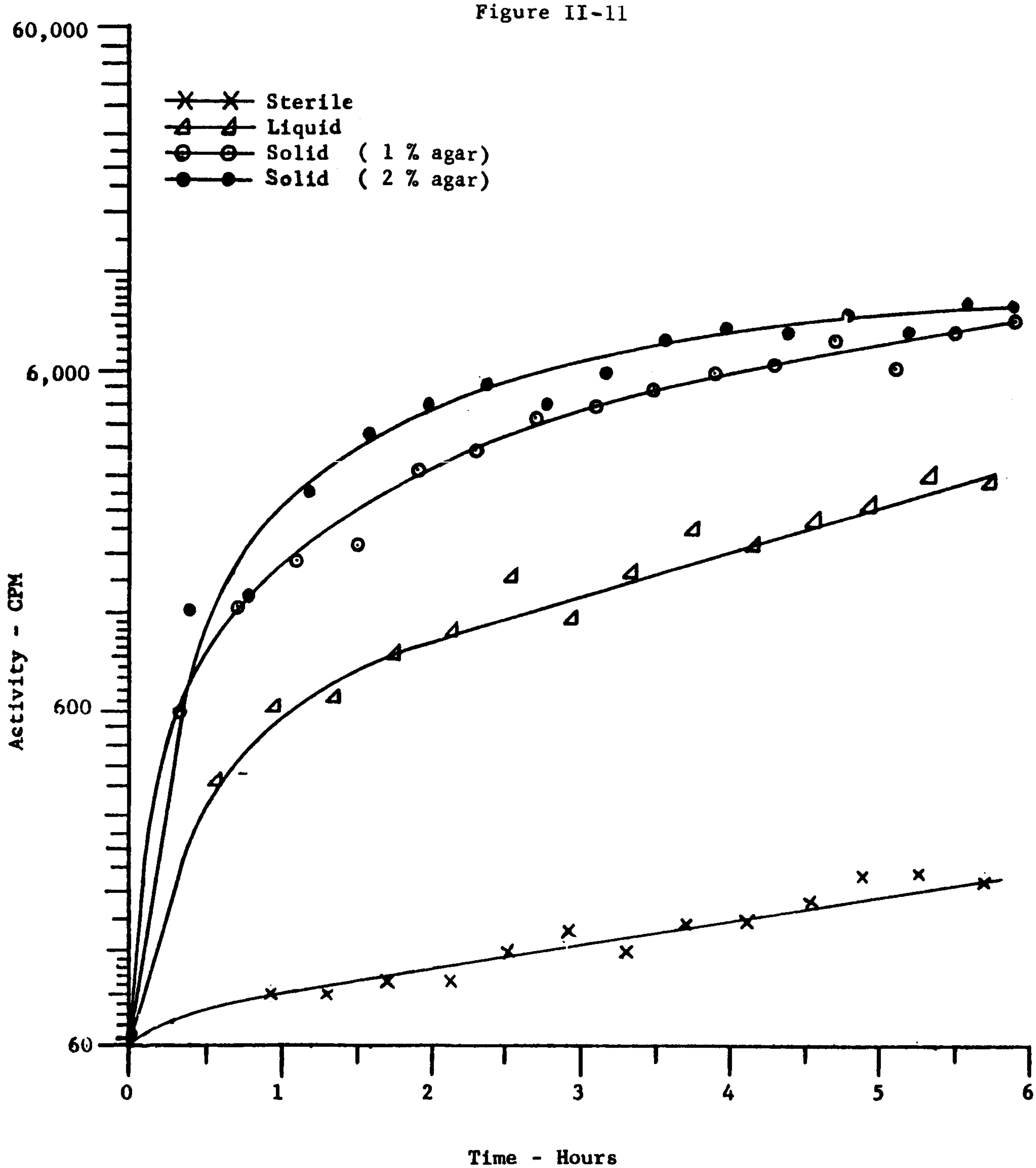
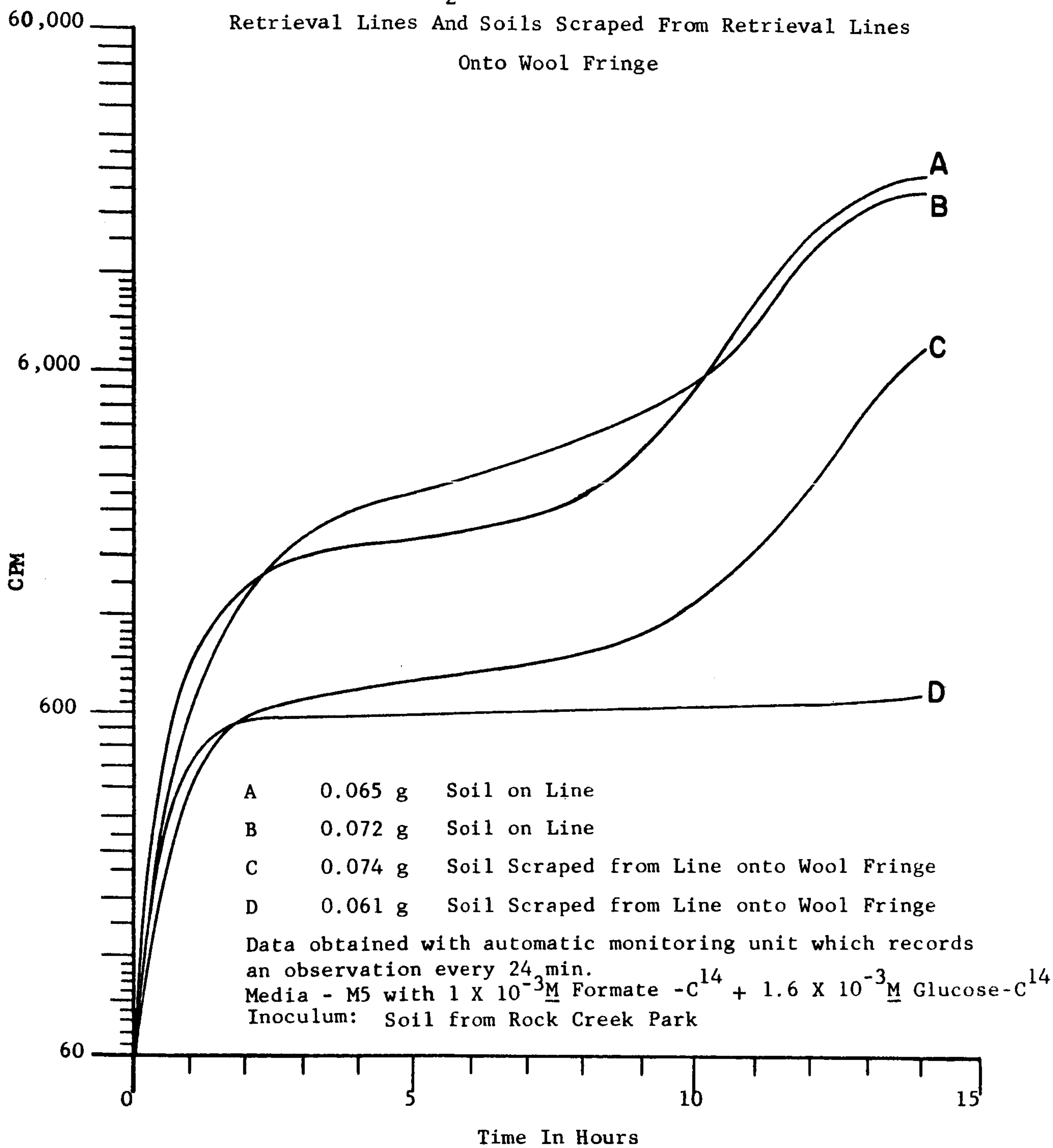


FIGURE II- 12

Comparison Of $C^{14}O_2$ From Soils Incubated In Chambers On Retrieval Lines And Soils Scraped From Retrieval Lines Onto Wool Fringe



the feasibility of using the string as support for the medium and provided guidelines for the present design of the instrument.

2. Protective Baffles for Cultivation Chamber

Related to the problem of attitude control, and associated with the use of the retrieval line as support for the media, is the need to prevent any medium from dripping onto the detector. To accomplish this, solid foam baffles were added to the regular metal baffle system. The material used was Scott Industrial Foam which will withstand sterilization and has the desirable characteristic of being impermeable to liquid but not to gases. Baffles of various porosities were tested and it was found that, in no case, were they detrimental to the test sensitivity. Foam baffles have been incorporated into the present instrument. They have been used in field tests in which the present instrument was successfully tested upside down.

A number of other factors have been examined in order to provide some guidance for instrument design. These included a comparison of CO_2 collector areas, some comparisons of the planchet method to the system using solid state or Geiger-Müller detectors, and a study of the effects of various amounts of retrieval line on detectable CO_2. It was found that the collector area can be a limiting factor since the use of larger collector areas resulted in an increase in detectable radioactivity. Other factors, primarily associated with the availability of adequate electronic components and the "state of the art" of detectors, will have to be considered before any area is ultimately selected.

In general, a comparison of the two incubation methods (planchet vs. instrument chamber) indicates that much sensitivity might be gained in a system similar to the planchet method if an adequate soil sample could be introduced. However, the incubation procedure cannot be dissociated from that of soil sample collection since utilization of a planchet-type procedure seems to require a method of soil removal from the retrieval line. When attempts were made in the laboratory to collect soil manually on retrieval lines and then transfer it to planchets, results were not as good as when the entire retrieval line was used in the culture chamber. When equal inocula were used results were better in planchets. Future design efforts will require further consideration of the relationship between the sample collection and incubation method.

Finally, it was found that too much retrieval line packed into a chamber could decrease the sensitivity of the system. The retrieval line is presently wound in a broad, flat manner to provide adequate space within the chamber.

H. FIELD TESTS

Field tests have been conducted with the second and third models (Figure III-2) of the instrument. The tests are designed to check instrument reliability and response sensitivity. The mechanical aspects of the tests are discussed in the section on Instrumentation. The biological responses have been positive and reasonably rapid. Responses from some of the early tests with Gulliver II were lower than desired but examination of the instrument revealed that in one test, which was conducted in freezing weather, the heating unit was not adequate to maintain the incubation temperature at a reasonable level. This failure has since been rectified. In a second test, it was found that LiOH used as a CO_2 collector became moist and diffused, a fact which may have resulted in the detection of less activity than was actually present. Even though there were some minor difficulties, both tests showed positive responses.

In a third field test, a rapid response was obtained with activity ranging from 120 cpm after the first hour to 32,300 cpm after 20 hours. Results of the first 13 hours are shown in Figure 13. Results of the first 12 hours of a further field test are shown in Figure 14. The response after 12 hours was 1500 cpm and after 21 hours rose to 5100 cpm. The curve is similar to others obtained from field tests.

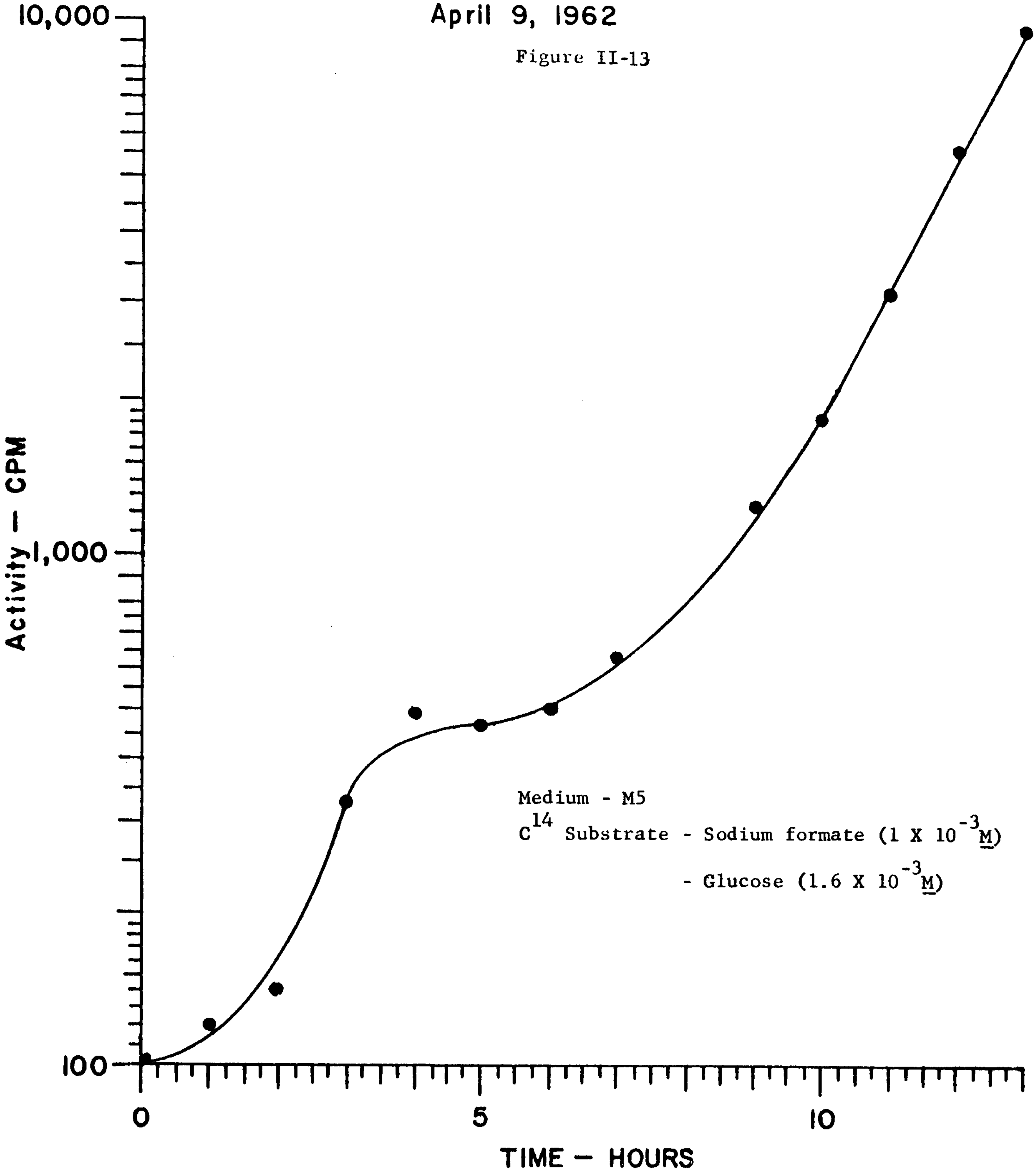
Mark III Gulliver has been field tested in a manner somewhat different from the second model.

Five tests were conducted with a single instrument. They were designed primarily for testing the mechanical aspects. In all other tests, two complete instruments have been tested simultaneously - both being run automatically by an industrial programmer. In the first two tests of the series using two units, both instruments were sterilized by autoclaving and carried into the field. One unit was cycled through the entire sequence of events; from firing of projectiles, which carried the soil retrieval lines, through to the counting of response. In the second unit the retrieval lines were not fired but were wound inside the chamber prior to sterilization. All of the other operations were carried out, and in this way the second unit served as a sterile control chamber. The tests were conducted on a clay field. The response from the second test is shown in Figure 15. The difference between the inoculated test instrument and the control chamber is very obvious. Activity at the end of 24 hours was 110,000 cpm in the test and 650 cpm in the control.

Field Test — Washington Sailing Marina

April 9, 1962

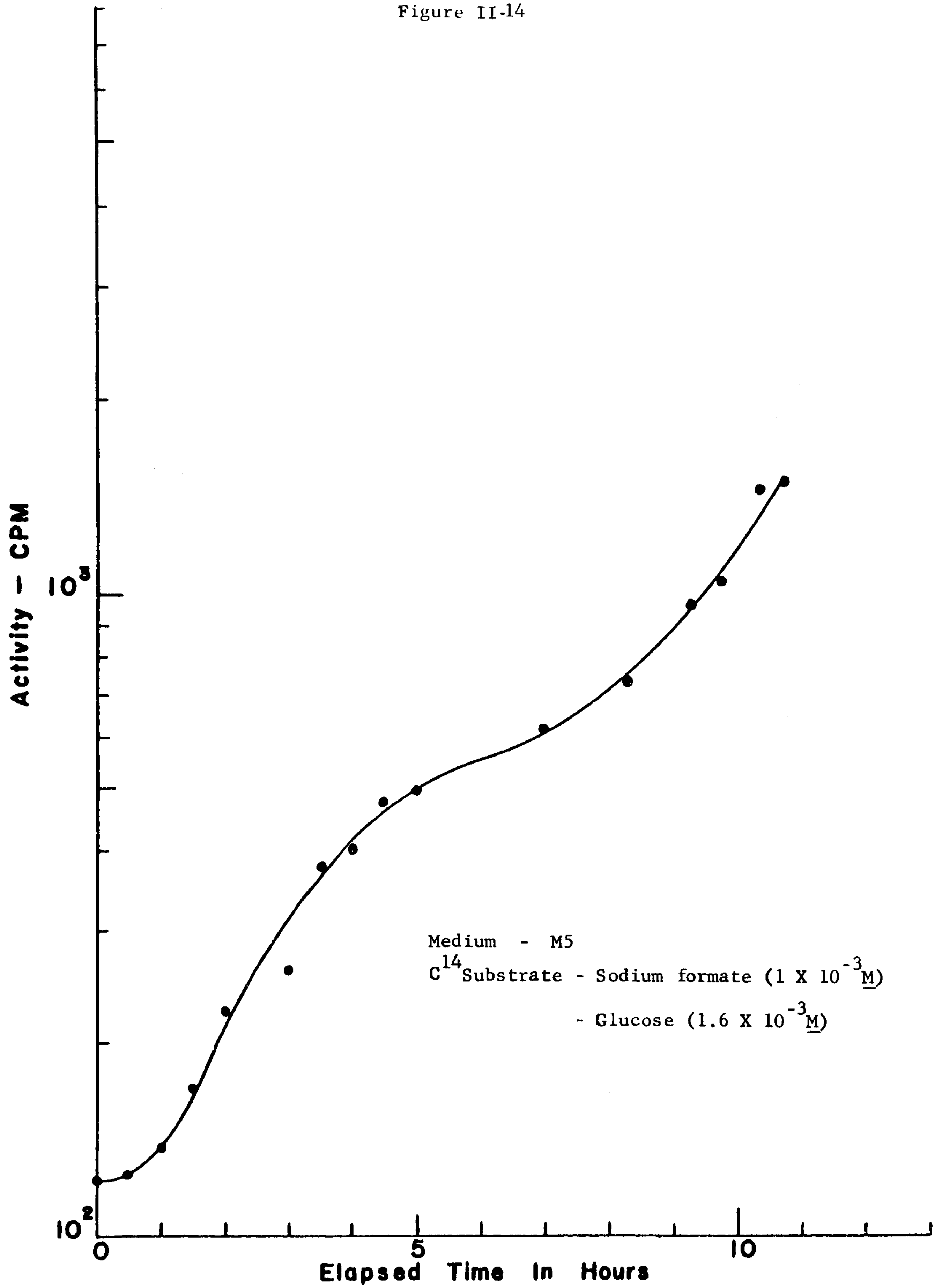
Figure II-13



Field Test – Washington Sailing Marina

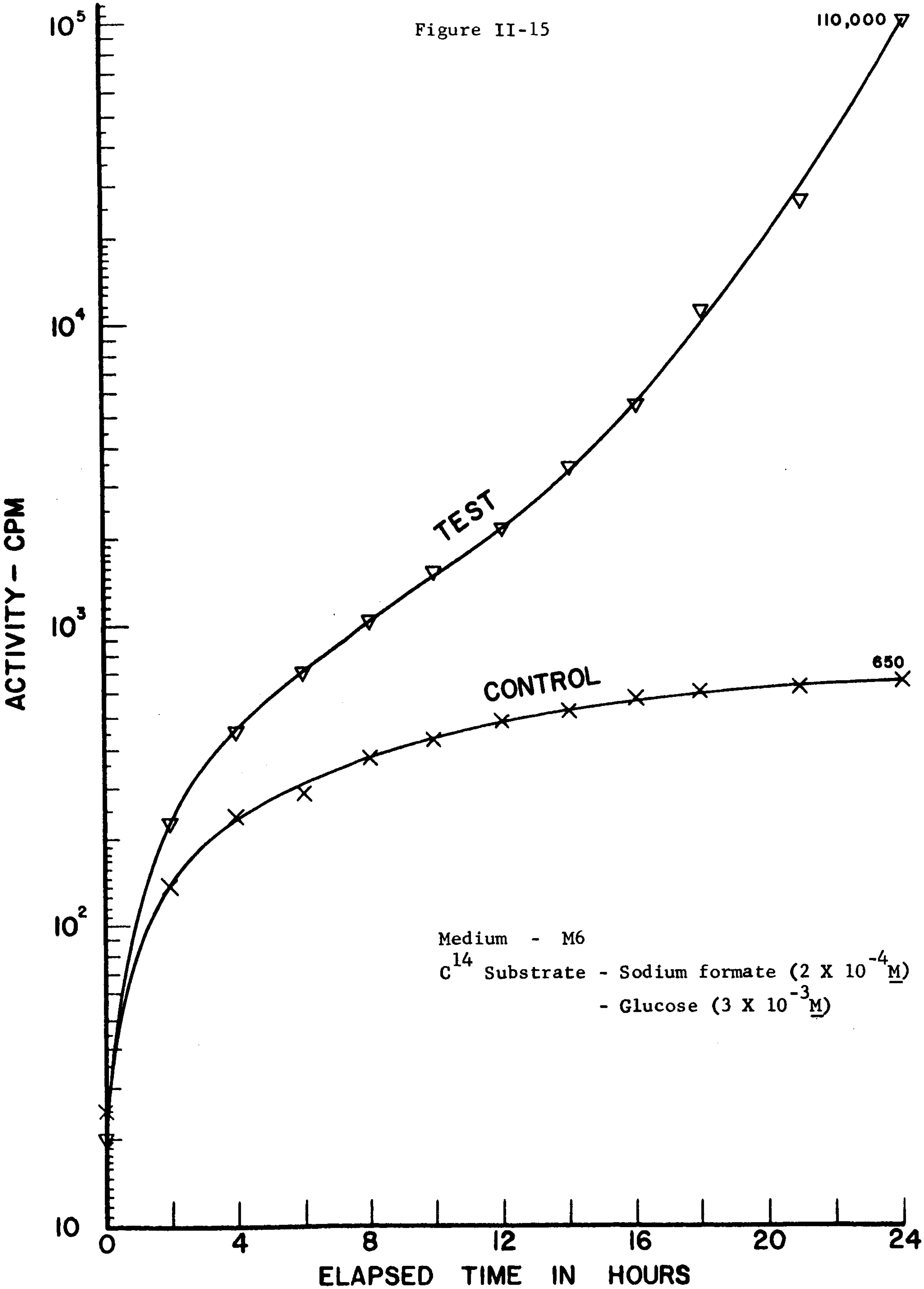
April 19, 1962

Figure II-14



FIELD TEST - NOVEMBER 27, 1962

Figure II-15



Medium - M6

C^{14} Substrate - Sodium formate (2×10^{-4} $\underline{M}$)

- Glucose (3×10^{-3} $\underline{M}$)

In three subsequent tests of Mark III, the units were inverted to test the omnidirectional capability of the instrument. Antimetabolite (Bard-Parker) was also included in both chambers. The first of these tests was only a partial success because a problem in the programmer caused several operations to misfire. In one instrument the entire cycle fired successfully with the exception of the antimetabolite which fired prematurely. In the second instrument, all operations fired simultaneously and nullified the test. The radioactivity from the first instrument was determined and found to be identical to previous sterile control curves. This indicated effectiveness of the antimetabolite. The problem in the programmer was corrected and two more field tests were carried out. Again both instruments were inverted to test the omnidirectional capabilities and both contained antimetabolite (Bard-Parker Germicide). All operations were completed successfully for both instruments. The first of these two tests was conducted on snow covered with a crust of ice. The sample appeared extremely light and the response was slow. Antimetabolite was fired into one chamber which remained at 600 cpm from 12 hours to 28 hours. The activity in the other chamber reached 8,000 cpm after 30 hours indicating a successful test. The second of these tests failed because the lines were siliconized excessively. The medium was not absorbed by the string and a leak developed around the baffle, contaminating the detector.

The final field test was conducted in cold ($3^{\circ}C$), windy, weather on frozen ground. After the programmed operations were completed and radioactivity detection was begun, the instruments were moved inside for further monitoring, (as yet, no automatic recording devices are included and monitoring requires the attendance of personnel). Activity was followed and, after

2.5 hours, antimetabolite was introduced into one instrument. The selection of the instrument to receive antimetabolite was based on previous field test results. It was decided to wait until both instruments reached 1000 cpm and then inject antimetabolite into the one which responded more slowly. Results are presented in Figure 16. The test was completely successful, and it can be seen that the activity in the control chamber remained considerably below the activity in the uninhibited chamber.

The field testing program has yielded a number of completely successful tests, some partially successful tests, and some failures. It has served to emphasize the strengths and weaknesses of the present instrument and thereby to provide guidelines for improvements. Extensive field testing, and necessary modifications are planned for the immediate future.

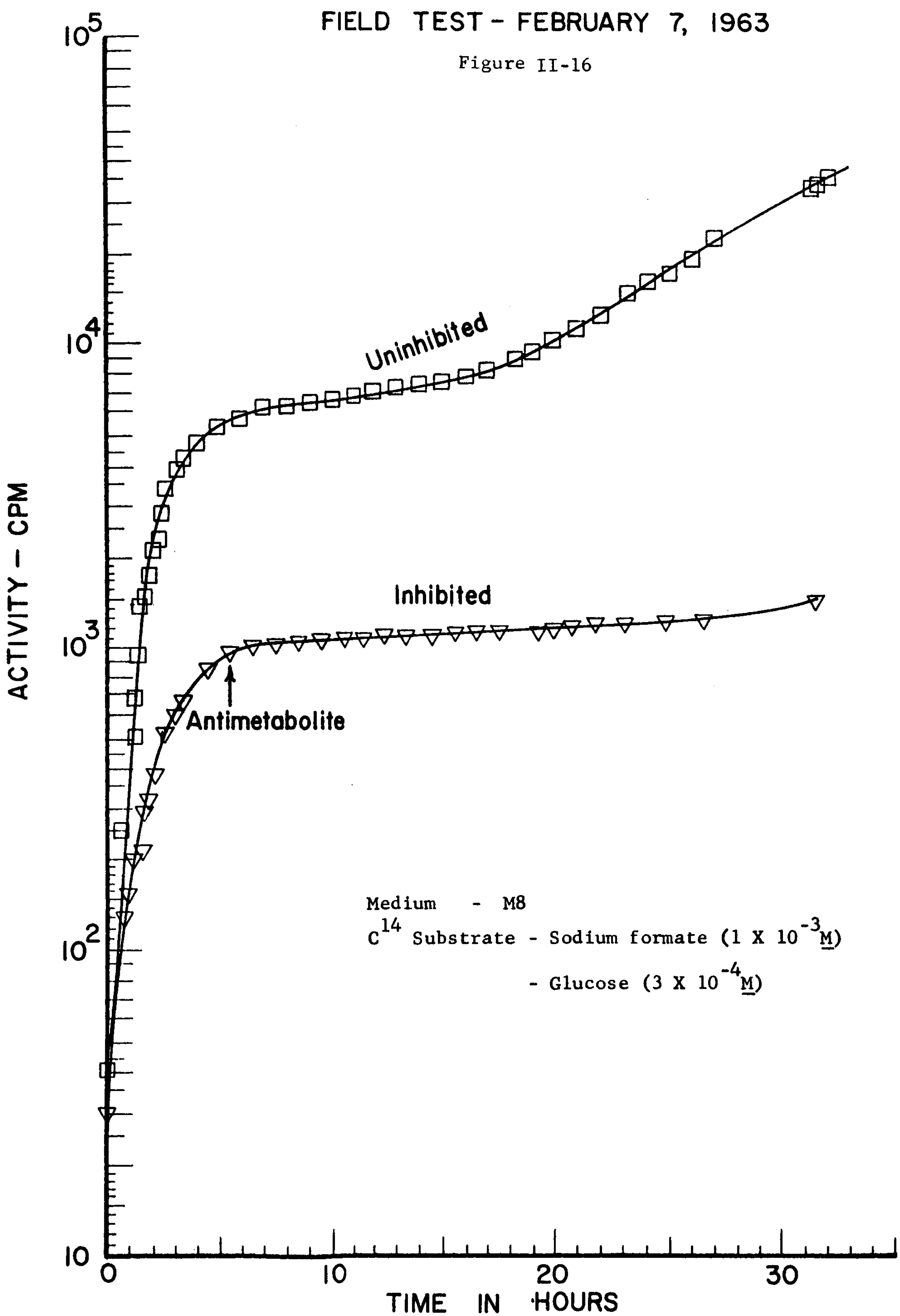
I. PERSONNEL

Dr. Norman H. Horowitz, Professor of Biology, California Institute of Technology, has become associated with the Radioisotopic Biochemical Probe for Extraterrestrial Life as an experimenter.

J. PUBLICATIONS

The design of the experiment and instrumentation of Gulliver have been published in the following journals:

1. Science 138: 114-121 (1962).
2. Nucleonics 20: 71-72 (1962).
3. Proc. 12th Lunar and Planetary Exploration Colloquium (May 1962).
4. Missiles and Rockets (October 8, 1962).


FIELD TEST - FEBRUARY 7, 1963
Figure II-16
ACTIVITY - CPM
10
10^2
10^3
10^4
10^5
0
10
20
30
TIME IN HOURS
Uninhibited
Inhibited
Antimetabolite
Medium - M8
C^{14} Substrate - Sodium formate (1×10^{-3} M)
- Glucose (3×10^{-4} M)

Second Annual Progress Report

NASr-10

February 15, 1963

Respectfully submitted,

Gilbert V. Levin

Gilbert V. Levin
Experimenter

Norman H Horowitz

Norman H. Horowitz, Ph.D.
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Allen H. Heim

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Senior Microbiologist

Mary-Frances Thompson

Mary-Frances Thompson
Biologist

LIST OF ILLUSTRATIONS

List of Illustrations (Cont'd) Page No.

PART III

INSTRUMENTATION

14 February 1963

AMERICAN MACHINE & FOUNDRY COMPANY
Research & Development Division
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Alexandria, Virginia

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Instrumentation Project
Engineer

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Sensor Department

I. PART III INTRODUCTION

A. OBJECTIVES AND ASSUMPTIONS

The objective of this program as outlined in the proposal submitted in November, 1961 to Resources Research, Inc. for inclusion in their proposal to the National Aeronautics and Space Administration was to develop in coordination with Resources Research, Inc. the automated instrument for radiobiochemical detection of microbial life into a more rugged, reliable and refined model which would more closely approximate a "flight" model. This general objective is tangibly represented in the current model of the instrument, named Gulliver III. It has been successfully tested in the field. A photograph of this instrument taken during a field test is shown as Figure III-1. A photograph of the instrument in an inverted position is shown in Figure III-2.

Certain assumptions on environmental and operational conditions were stated in the proposal based on the knowledge that was known or had been made available about the Mars flight program. These assumptions were to be used as design guidelines until specifications and requirements became available. Some of those existing state-of-the-knowledge assumptions have proven to be adequate but others were not compatible with specifications and requirements that became available from the Jet Propulsion Laboratory. The most notable of these assumption discrepancies were as follows:

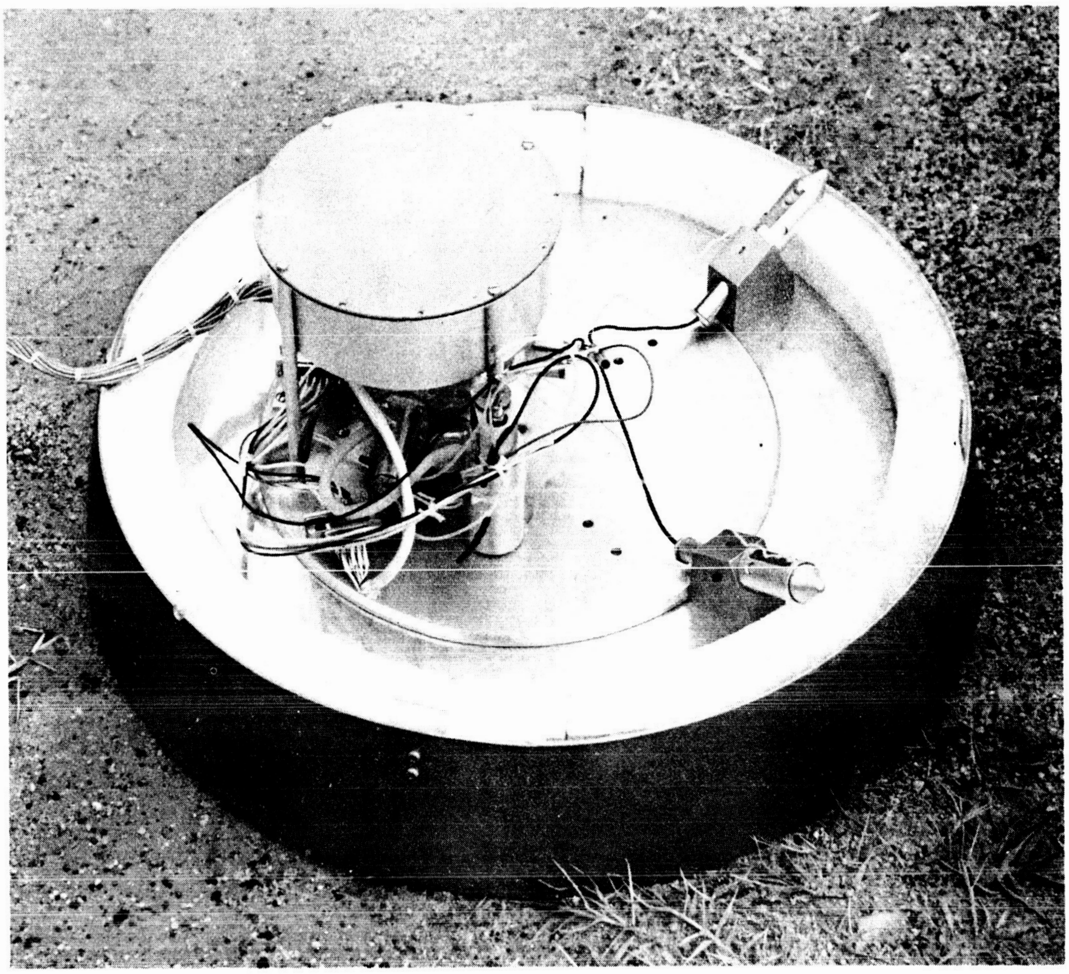


Figure III-1. Gulliver III ready for field test.

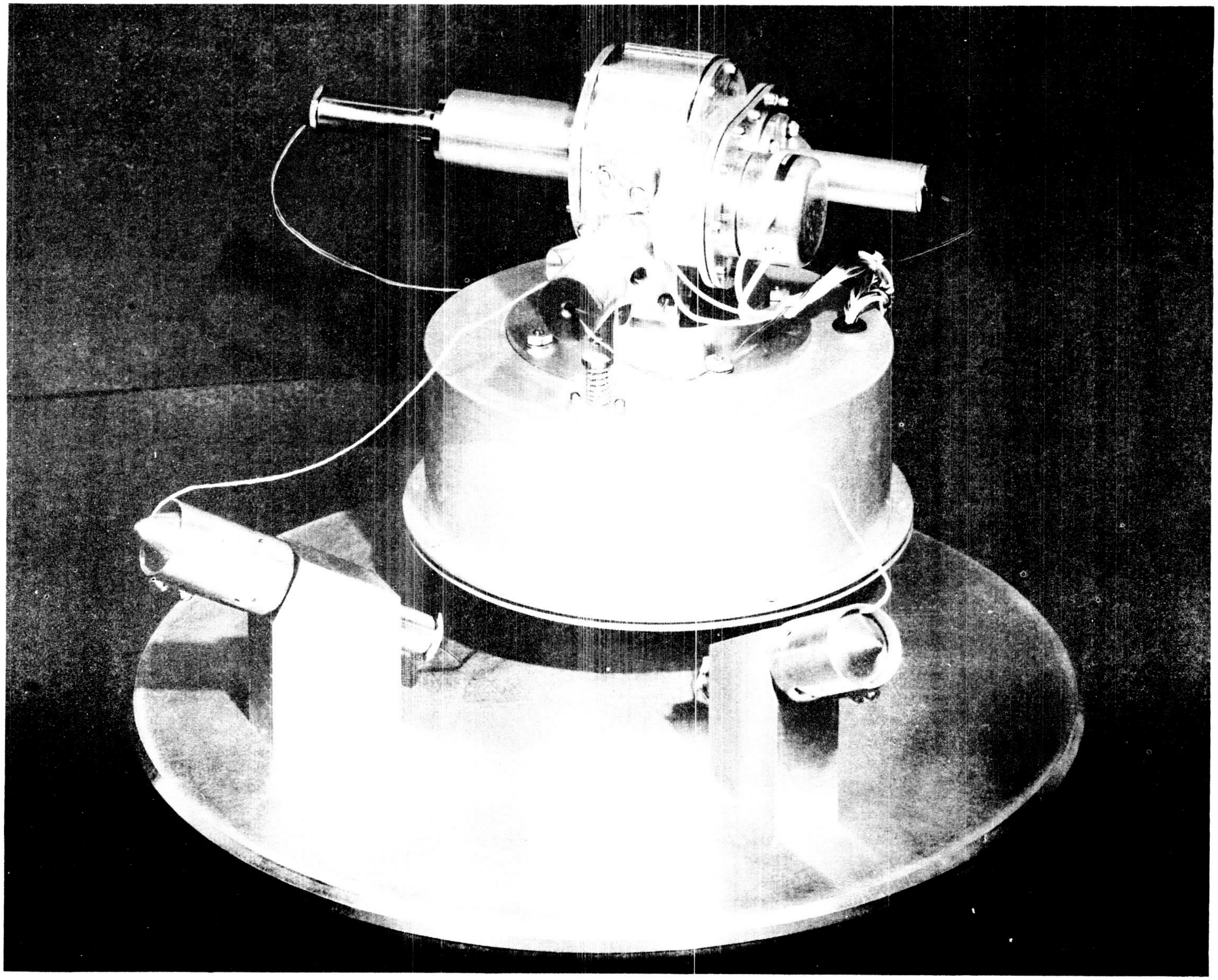


Figure III-2. External configuration of Gulliver III, inverted position.

1. It was assumed that the capsule after landing would be oriented within 30° of a predetermined gravitational axis. It was learned that no provision of attitude control by the capsule should be anticipated. This required a complete redesign of the mechanical system since gamballing was judged to be impractical for a combined all-degrees of freedom system.

2. It was assumed that preflight sterilization would be effected by dry heat of 125°C for 24 hours. It was learned that heat sterilization would be effected by 135°C for 26 hours and approval tests would be three cycles of 145°C for 36 hours. This had a strong bearing on changing from solid state radiation detectors back to geiger detectors.

3. It was assumed that limited pressurization of 10^{-5} mm H_g would be maintained during flight. It was learned that the instrument would be exposed to space vacuum during flight.

4. It was assumed that the capsule would have all the necessary scaling, logic, and data handling capabilities for control of this experiment. It was learned that the capsule would have a timer that would provide the signals to actuate the electromechanical elements of this instrument, hence developing a separate programmer was not necessary. It was learned that the capsule would not have counters or scalers to store or to convert the output signals from the Gulliver radiation detector. Nor was there expected to be a capsule capability of receiving command signals from earth which would enable human decisions to control the time of antimetabolite injection.

This change in plan required a study of electronic techniques to accomplish the necessary logic and data handling functions.

B. FACTORS AFFECTING PROGRAM DIRECTION

The model of the instrument which was developed and field tested to demonstrate feasibility in 1961 and early 1962 was judged to be satisfactory for consideration in a Mars flight program as far as size, weight, and power were concerned. However, sensitivity, reliability, and environmental ruggedization required much improvement.

In the area of mechanical design, probably the most significant factor that changed the configuration of the instrument from that existing at the first of the year was the requirement that the instrument had to operate satisfactorily with no foreknowledge as to the gravitational axis. Since handling of liquids that are transported in sealed ampules is crucial to successful operation, methods had to be devised to perform these functions. The technique employed in Gulliver III seems obvious and simple at first glance, but this concept followed many other schemes which were postulated, analyzed, compared, and rejected.

The trips and conferences in February, March, and May, when this experiment was discussed with personnel of the National Aeronautics and Space Administration and the Jet Propulsion Laboratory, uncovered many specifications and requirements for the 1964 Mariner B capsule which were not known at the time the proposal was written and when this contract was started.

When it became apparent that this experiment was considered for inclusion in the 1964 Mariner B it necessitated that the development effort be greatly accelerated to meet delivery schedules for breadboard and prototype models. Although need for acceleration of the schedule was painfully obvious, definition of some of the interfaces and requirements of the capsule and some of this instrument's interfaces and requirements were lacking. This was further complicated by the knowledge that the instrument sensitivity needed to be improved and that the degree of improvement attainable by different design changes could not be determined until numerous interrelated tests were conducted. Other tests also had to be conducted to determine whether the parts or materials of a design would withstand some of the more obvious severe environments -- most often high temperature. Requirements for reliability and reproducibility also dictated that numerous other tests be performed.

When this project was definitely assigned to the JPL Mariner B capsule program, a request for additional funding to provide a new feasibility demonstration system by November 23, 1962 was submitted. Before this date arrived the program for a capsule for the 1964 Mariner B was dropped. At that time the effort was redirected away from hardware and environmental and reliability testing towards more basic investigations to improve instrument sensitivity. These more basic investigations included study and test of sensitivity of detectors other than geigers, improvement of the sample collection technique, and determination of requirements for removing non-metabolic tagged gases from the broth before incubation is started.

II. MECHANICAL AND CHEMICAL DEVELOPMENT

A. MECHANICAL EVOLUTION

Once the mechanical design for Gulliver III was conceived, the system was broken down into functional subsystems. Mockups of the mechanical subsystems were fabricated and tested to assure that they operated properly before they were incorporated in a detailed design of the total system. These mockups included: a very simple incubation chamber with a hollow perforated spool on which sample collection lines could be wound, a breaker for rupturing the broth ampule in the spool, baffles between the incubation chamber and gas collector and detector, and holes for string ports and for introduction of flushing gas or antimetabolites; a string port closing mechanism; a baffle opening mechanism; a line retrieval mechanism using the rotary solenoid with pawl and ratchet for driving the spool; a CO_2 gas generator; and linear mechanical actuation device using explosive squibs instead of the similarly pyrotechnic but non-explosive bellows motors.

After these subsystems were tested, the design was firmed, and detailed drawings were made of the current Gulliver III instrument. Two units were built initially, and these were successfully demonstrated November 27, 1962. A third unit has since been fabricated.

B. GULLIVER III, DESCRIPTION AND OPERATION

Figures III-3 and III-4 which appeared in the quarterly report of August 1962 show the layout of the Gulliver III instrument. Figure III-5 is a current

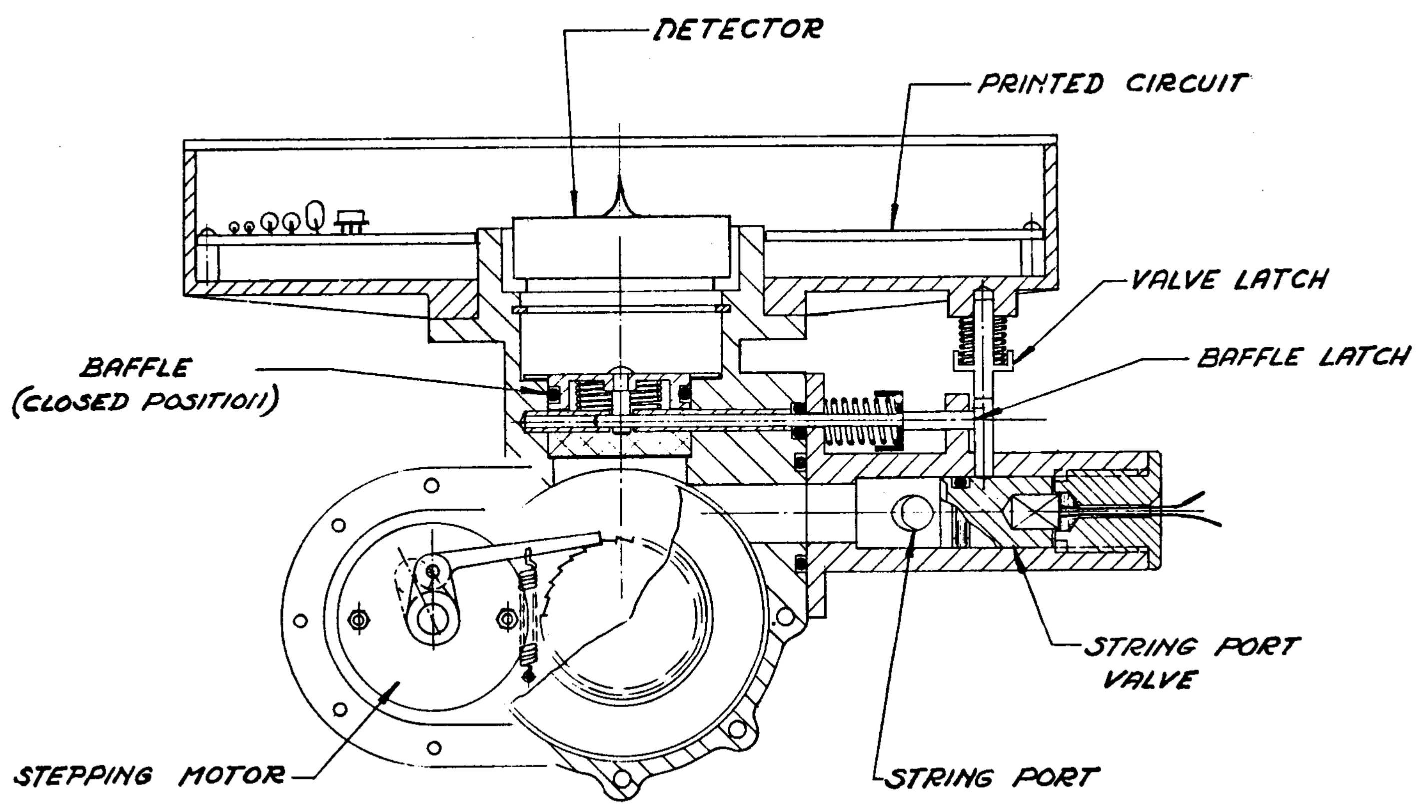


Figure III-3. Front elevation, cutaway of Gulliver III.

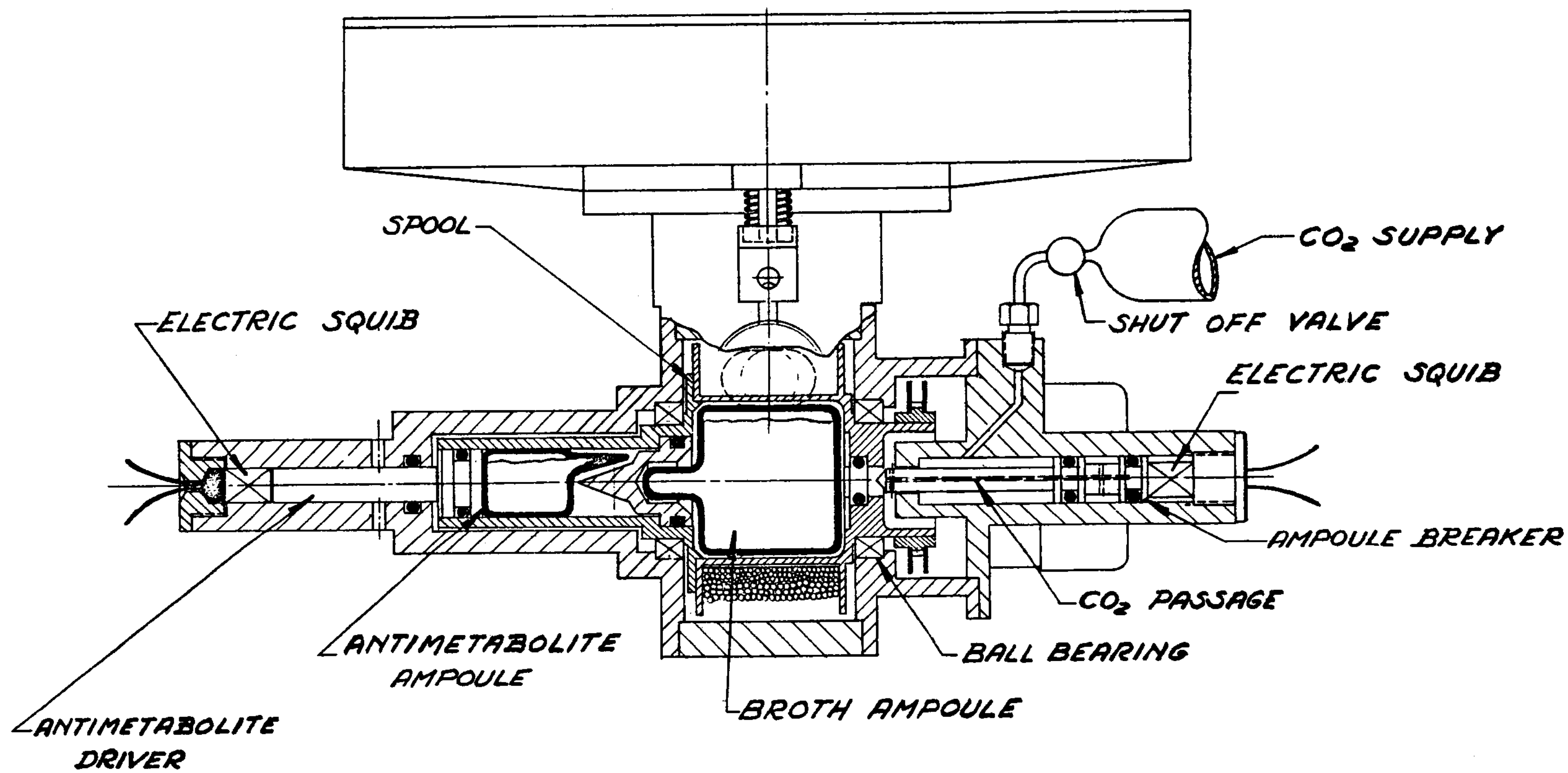


Figure III-4. Side elevation, cutaway of Gulliver III.

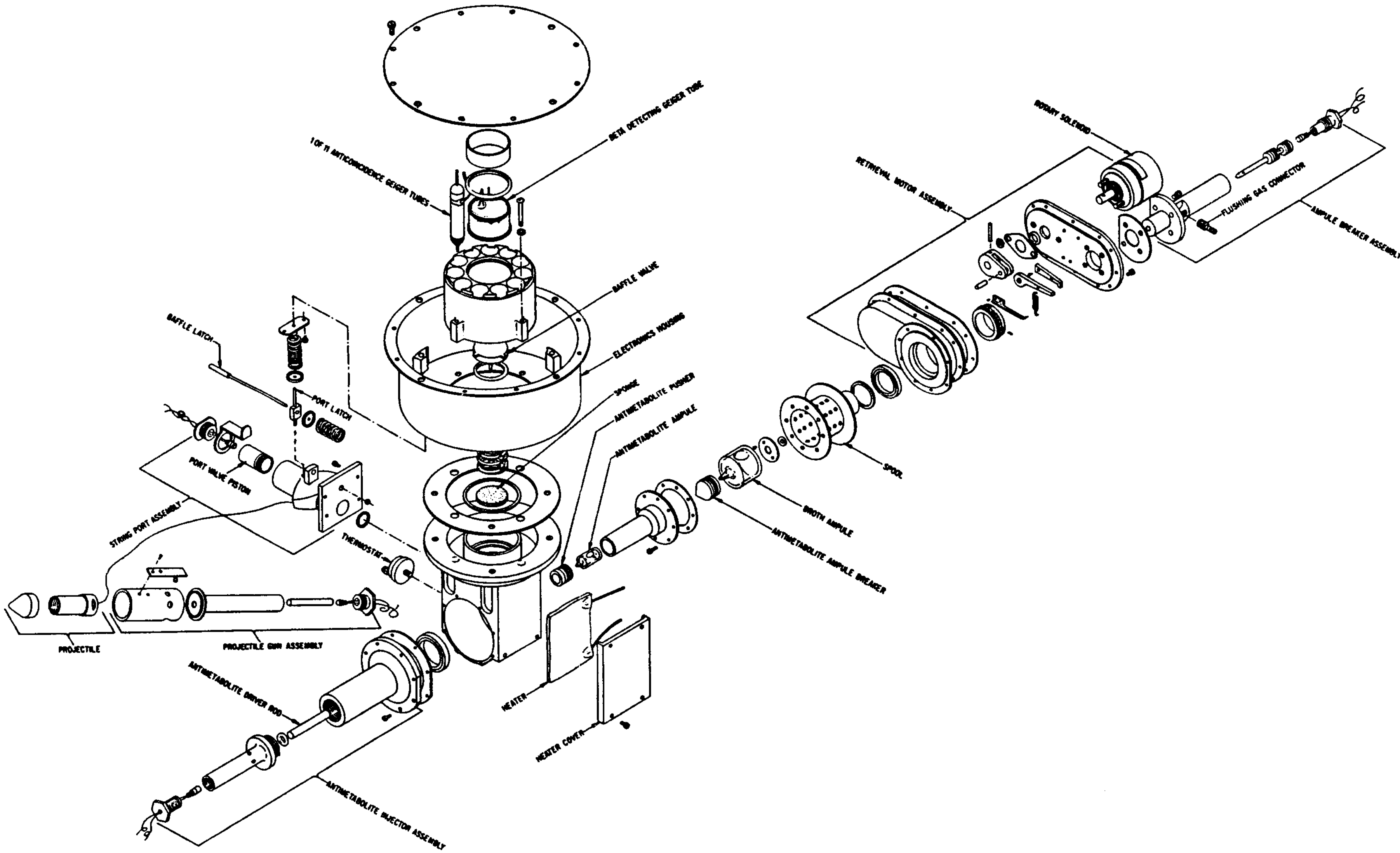


Figure III-5. Isometric assembly drawing of Gulliver III.

isometric drawing of the instrument as the design now exists. It will be noted that there are a few minor mechanical differences between the two drawings. Also, no specific detector is illustrated in Figure III-3, but in Figure III-5 the C^{14} detecting thin window geiger tube (Amperex 18515) and the eleven anti-coincidence guard tubes (Amperex 18550) are shown along with their mounting block.

For purpose of understanding the operation of the instrument the reader may refer to Figures III-3, 4 and 5. The first function of the instrument is to fire two projectiles each containing approximately 25 feet of silicon greased cotton string. The projectiles over-travel the 25 feet and free the lines which then fall to the ground. One end of each line passes through a string port and is attached to the spool in the incubation chamber. At the time the projectiles are fired the rotary solenoid is actuated and the lines begin to wrap onto the spool. As the lines drag across the ground they collect loose dust and small granules of soil. It currently requires approximately 2 1/2 minutes for the two lines to be retrieved.

After the lines are on the spool the broth ampule is broken by the ampule breaker which is coaxially lined with an "O" ringed opening on the motor end of the spool. With the ampule shattered the spool continues to be rotated for about 30 seconds to aid distribution of the broth into the lines and to dump broth out of any large ampule fragments that might hold broth. A thin layer of filter paper, not shown in the figures, is wrapped directly on the outside of the spool and lies under the collection lines. This filter paper minimizes any splashing if the ampule rupturing is violent, and it serves also to minimize drippage if the lines do not cover the perforated spool smoothly from end to end.

After the spool stops rotating the traces of nonmetabolic gas are flushed out by means of a purging gas which is introduced through a coaxial hole in the ampule breaker. The gas supply end of this hole terminates between two enlarged "O" ring sealed sections on the ampule breaker. The forward of these two enlarged portions serves to stop the ampule breaker travel; the rear "O" ringed portion prevents the gases and powder from the squib from entering this gas passage. A small hole through the wall of the cylinder of the ampule breaker vents the burnt powder and gases when the breaker nears the end of its travel. The connection to flushing gas supply is mounted on the side of the ampule breaker section. Thus the flushing gas passes through the opening in the side of the wall, through the ampule breaker, out through the broth on the sample collection lines on the spool, and on out through the string port to the outside atmosphere.

After the flushing has been completed and a waiting period has elapsed to permit the atmosphere in the incubation chamber to equilibrate with the outside atmosphere via the string port, a squib is fired which drives the "O" ring sealed piston type valve which seals off the string port and cuts off any line which might still be in the port if by any circumstances the line was not completely retrieved. When the rear of the piston valve passes a given point in its travel the burned powder and gases from the squib are vented to the atmosphere and a spring loaded latch through the side of the wall is permitted to drop in behind the piston. When this valve latch drops it permits another spring loaded latch to be released. This baffle latch in turn releases the spring loaded baffle-valve that has sealed the incubation chamber from the radiation detectors and gas collector.

At this point the system is ready to start monitoring the gases collected by the collector on the face of the radiation detector.

It should be noted that with the detector and gas collector sealed in position the baffle-valve prevents the gas collector from collecting CO_2 from the atmosphere before the experiment starts and from picking up nonmetabolically evolved gases during the flushing operation. Its other principal function is to prevent line of sight between the radiation detector and the tagged broth on the lines in the incubation chamber since the detector could not distinguish between a C^{14} beta coming from the broth and a C^{14} beta from a CO_2 molecule which was metabolically evolved and collected. To further eliminate line of sight between the detector and broth and to prevent any broth drippage in the incubation chamber from getting down into the detector if the instrument is inverted with the detector below the incubation chamber, a sponge which freely passes gases but is not wetted by broth and prevents its passage is secured in the throat between the baffle valve and incubation chamber.

To inject the antimetabolite, a squib is fired which pushes a small driver rod into the section containing the antimetabolite ampule. This rod pushes on a piston which pushes the ampule against a tight fitting pointed piston which breaks the ampule. Before the driver rod reaches the end of its travel and vents through a small hole in the wall, the ampule pushing piston has pushed everything before it into the center of the spool in the incubation chamber. There the antimetabolite will be taken up by the lines and mixed with the broth to stop metabolism.

It should be noted that a heater pad is mounted on one outside of the incubation chamber and a fixed temperature thermostat is mounted on the opposite wall. A cover is placed over the heater to cut down on heat losses.

C. SAMPLE COLLECTION

Two primary objectives were pursued in developing a sample collection system: (1) a reliable method for collecting samples beyond the immediate periphery of the capsule; (2) a maximum soil sample collection that would not interfere with the biological and radiological functions of the instrument.

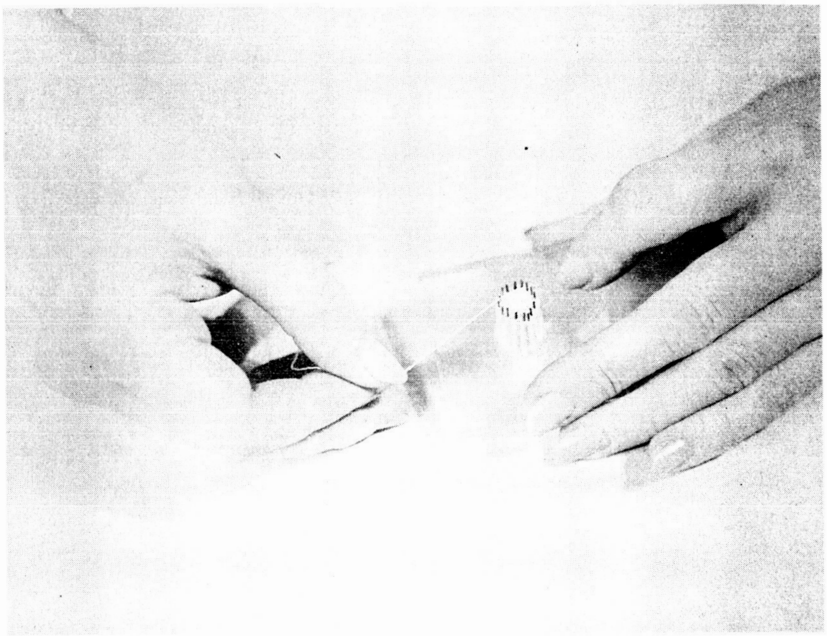
1. Projectiles and Squibs

The earliest system for deploying the sample collection line consisted of winding the line in a spiral configuration and loading into a cylindrical projectile with a small hole in the rear for the pay-out. Several variations in winding configuration were tried and a fairly dependable method was achieved for a 27 foot line. Refinements were made on the gun and projectile combinations to reduce its weight and improve simplicity and dependability. Complete discussion of these early efforts are found in Progress Reports No. 5 and 6 of May and August 1962.

The reliability of the spiral winding configuration was not considered great enough so a new method was developed. The wrapping of the string is controlled by a wrapping fixture shown in Figure III-6, consisting of sixteen .032 diameter rods spaced equally on the circumference of a .437 diameter circle. The actual wrapping is done by looping the string over the pins, back and forth in one direction until all pins are used, never letting any two segments of the wrapping cross. After wrapping one layer the direction of wrap is changed ninety degrees and a second layer is done the same as the first layer. On changing layers of wrappings care has to be used to not loop the string over itself. The procedure is continued until the entire length of the line is used. The wrapped line is then forced into the projectile by a plate which slides along the rods. This is illustrated in Figure III-6.

To test the reliability of this system seventy six lines were wrapped, loaded and fired. The results of these firings: seventy four successful shots with all the chenille and thread unwinding and leaving the projectile without snarls or knots. Two shots failed because of human error in rigging the gun.

Based on the test performed, this method of wrapping shows possibilities for a high reliability figure.



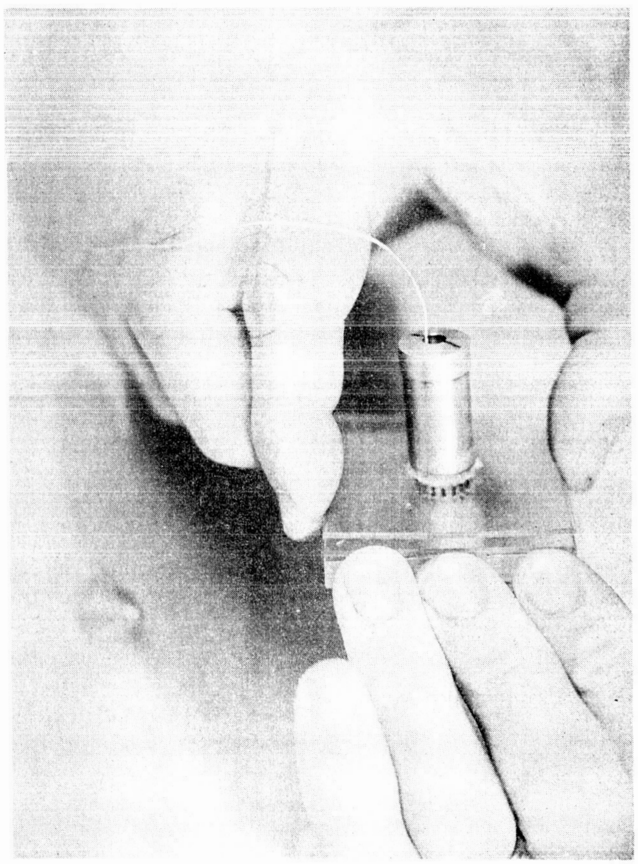


Figure III-6. Top: Line being wrapped on fixture. Bottom: Wrapped line being loaded into projectile.

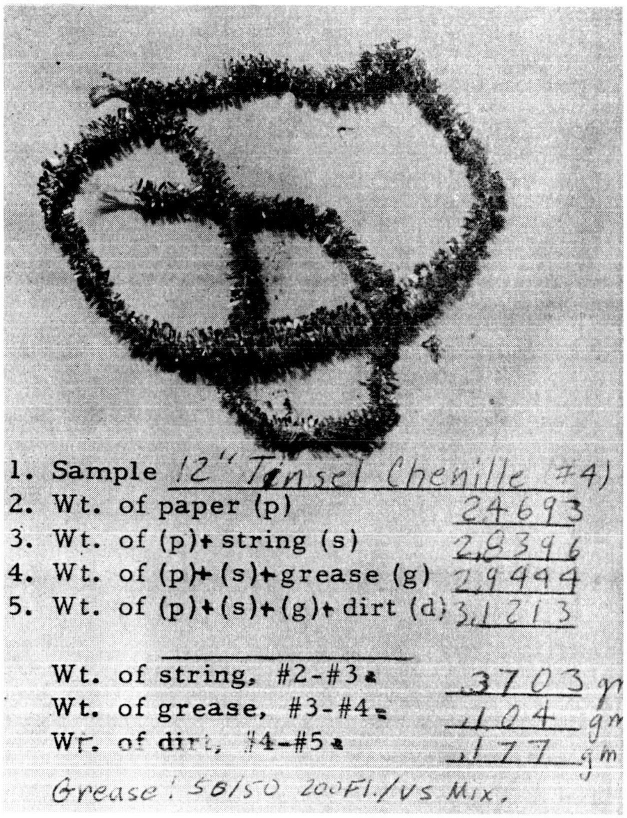
2. Collection Lines and Sample Size

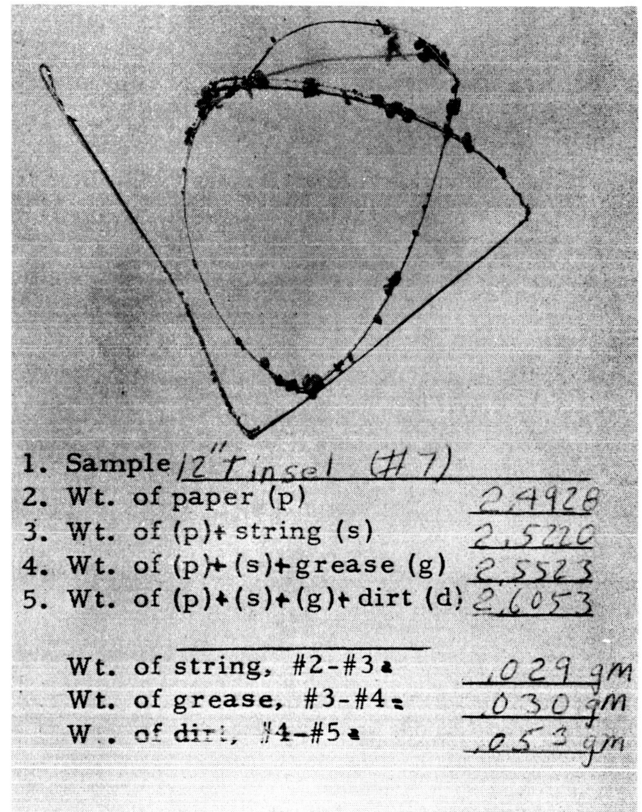
To maximize soil sample collection a two-part investigation was made of sample collection materials. The first part was to study effects of mechanical configuration of collection lines on the size of sample collected. The second part, to study the effects of various types of silicone grease adhesives on the size of sample collected. These advantages of using the silicone greases are: (1) biological inertness; (2) low vapor pressure; and (3) little change of viscosity with temperature.

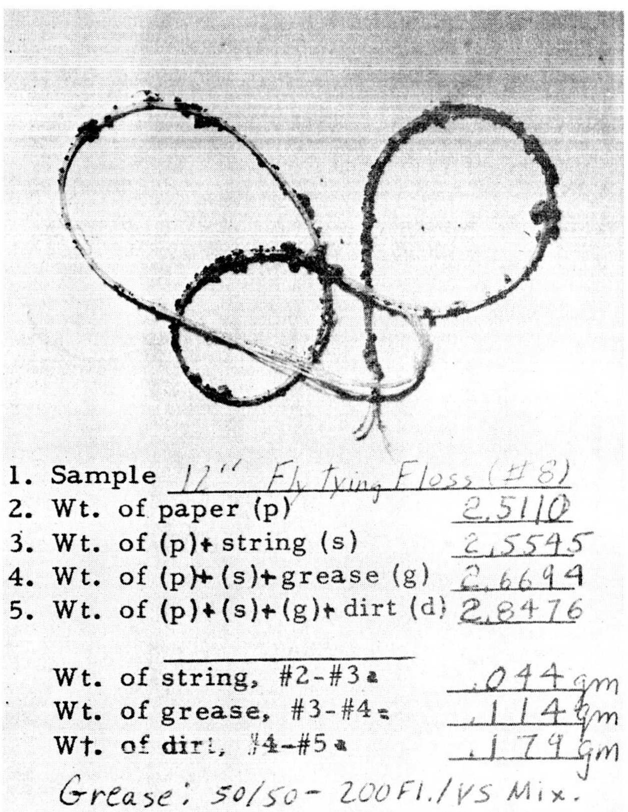
The mechanical investigation began with a test of chenille and line samples of various types. Some of the results are shown in Figure III-7. Each sample was weighed on electronic milligram balance, greased and weighed again then dragged a uniform distance across a controlled plot of earth and weighed again.

The following results were obtained:

No.	Sample	Wt. of Grease (mg)	Wt. of Dirt (mg)
1	Large White Cotton Chenille	878	143
2	Medium White Cotton Chenille	408	47
3	Small White Cotton Chenille	297	74
4	Tinsel Chenille	104	177
5	Spun Fur	215	113







Field test sample

Figure III-7. Samples collected on various types of line.

6	Wooly Yarn	253	81
7	Tinsel	30	53
8	Fly-Tying Floss	114	179

Two deductions are made from this data: First, from the dirt collected on the tinsel (#7) and Fly-Tying Floss (#8), relatively large particles of dirt are readily picked up by a simple line with sufficient grease applied. (Figures C1 and C2). Second, from the tinsel chenille sample (#4) small particles of dirt are readily picked up by the stiff, bristly arrangement illustrated in Figure III-8.

To explore further the possibilities in chenille configuration a visit was made to the Danville Chenille Company in South Danville, N. H. where methods for making chenille were studied. It was found that a wide variety of configurations were possible. One that showed particular promise was a spiral arrangement in which rows of cross-fibers were widely spaced. The possibility of introducing a soft wire winding on the core of the chenille for adding weight was also demonstrated. A variety of promising combinations were contracted to be made which did not arrive in time to be tested and include results in this report.

The following adhesives were tested for suitability as sample collectors: The General Electric SR-585, SR-529 and SR-520 Silicone Adhesives, and the Dow Corning Valve Seal and 200 Fluid (@ 200, 000 centastores).

The silicone based adhesives showed little advantage over the silicone greases and were also judged to have a high risk of being toxic. Of the silicone greases the higher viscosity 200 fluid displayed superior sample collecting properties but caused the line to stick in the projectiles in two out of four firing tests. The valve seal silicone displayed good sample collecting qualities when properly applied to the lines in sufficient quantities. It was observed that the cause of inadequate sample collection in earlier field tests in which this grease was used was the fact that much of the grease applied had been wiped off the line during the wrapping procedure. A remedy for this was found in applying the grease after the line was loaded into the projectile. Some reliability of this method of greasing was indicated by seven test firings in which all lines were freely ejected from the projectiles.

The major limitation in sample collection using silicone grease as the adhesive is the fact that the larger the quantity of grease the greater the tendency for the broth to seep through the lines and drip out.

Limited tests were carried out to determine the limits of grease quantity that would insure no drippage with the approximately 3.5 ml of broth being used for field tests. In one field test where approximately 200 mg of grease was applied per projectile a prohibitive amount of seepage occurred and ruined the test. In a second field test where approximately 75 mg of grease was applied no seepage was observed. Further tests in this area are recommended. The lower right photo in Figure III-7 shows the sample collection lines after being removed from the instrument which showed rapid growth after the field test of February 7, 1963.

D. RETRIEVAL MOTOR

The retrieval motor for the Gulliver instrument had four primary requirements to meet: (1) to provide sufficient torque to draw the collection line in over rough terrain; (2) to provide sufficient speed to draw the lines in quickly; (3) to have a sufficiently low power requirement not to exceed available power supply; (4) to create no stray magnetic fields. A rotary solenoid (Ledex El, G. H. Leland Company) driving a pawl and a ratch wheel was tested and modified to give the following performance. At 20 pulses per second the spool turns at 30 RPM and brings in a 27 foot line in two minutes and thirty seconds. Torque at this rate is 5.75 ounce-inches. The average power drain at this rate is 14 watts for a 50% duty cycle. Since there are no permanent magnets in this system no stray magnetic fields are created. A more detailed discussion of this system is found in Quarterly Reports No. 6 and 7 of August and November 1962.

This system has performed satisfactorily in fourteen field test runs in a wide variety of weather conditions. In only one test did the motor fail to function properly and modifications were made to correct that weakness. The system withstood 250°F sterilization temperatures without effect on its function. To further examine possibilities for reliability in this system an investigation was carried out to find a stepping motor with the non-magnetic properties and efficiency required for this purpose. The Size 11 Variable Reluctance Stepping Motor manufactured by IMC Magnetics Corporation, Western Division was found to satisfy these requirements.

E. METABOLIC GAS COLLECTION

The metabolically evolved tagged gases from the incubation chamber of Gulliver are retained within the confines of the system by sealing off the string ports. But in order to achieve the maximum sensitivity, these gases are then collected where they can all be detected, on the face of the geiger tube itself. Ideally, every molecule of tagged gas given off by the organism would be deposited on the geiger tube face and would thereby furnish evidence of biological response by adding to the count rate.

In order to approach this ideal of 100% efficient gas collection, experiments were conducted with various compounds and agents to observe on a comparison basis the effectiveness of each. Agents to collect the gases and materials to bond the agents to the geiger tube face were given thorough investigation.

To test the gas collectors a source of $C^{14}O_2$ was required. The method of generating $C^{14}O_2$ was to place a mixture of $BaCO_3$, $BaC^{14}O_3$, and powdered citric acid in a weighing bottle which was set in a gas tight metal box. Water was introduced into the mixture via a burette sealed in the top of the box. Upon being wetted, the carbonate and acid reacted to produce $C^{14}O_2$. Thus, by placing gas collector samples inside this gas-tight box, uniform exposure to $C^{14}O_2$ was attained for each sample. Counting the samples in a gas proportional counter then furnished a comparison of the relative effectiveness.

Early investigation showed $Ba(OH)_2$ more preferable as a CO_2 absorber than activated charcoal or molecular sieves under conditions of high relative humidity. These latter "getters" became saturated with gases other than the desired CO_2.

Attempts were made using silk screen techniques to coat collector materials in adhesives in a uniform manner on flat surfaces, in order to develop a method of coating geiger tube windows with $Ba(OH)_2$. Adhesives used were Bondmaster M683 (160 hardener), RTV-102, Elmer's Epoxy-Metal Compound (Borden Chemical Co.) and Bostik adhesives Nos. 4045, and 4585. The Bostiks were poor adhesives for this purpose. Coating was accomplished by mixing adhesives with $Ba(OH)_2$ which had been sifted though a 160 mesh screen. The adhesives were then mixed as necessary with appropriate solvents in order to obtain viscosities suitable for the silk screen process.

Elmer's Epoxy-Metal Compund was judged the best of the adhesives tested. Additional investigation is required, especially of those adhesives listed in JPL Specification No. 30257.

Three general methods of depositing $Ba(OH)_2$ were used: mixture with adhesive before coating as described above, spraying the $Ba(OH)_2$ powder onto Krylon which had been sprayed immediately before, and deposition of a saturated methanol -- $Ba(OH)_2$ solution from which the methanol quickly evaporated. With all techniques it is important that the time between preparation and use be kept to a minimum or that the procedure be carried out in a CO_2 - free atmosphere, in order to minimize effects of atmospheric CO_2 absorption. In all tests, pads wet with a saturated $Ba(OH)_2$ water solution are used as references for collector sensitivity. The alcohol solution method holds particular promise.

A recurrent difficulty in all gas collection performed was the inability to obtain reproducible results. Even the variation among replicates was much higher than expected. A sub-contract was transacted with Dr. W. E. Schmidt of the Chemistry Department of the George Washington University to study this problem.

Dr. Schmidt utilized a technique that involved such improvements as preparation of samples in a dry box that contained both anhydrous calcium chloride and ascarite. A mixture of uniformly tagged $BaCO_2$ was then prepared. A small fan was placed in the gas exposure chamber in order to help diffuse the $C^{14}O_2$ uniformly.

These precautions improved the reproducibility to an average deviation of from 13% to 17% for four replicates. Utilization of this technique in the future will allow gas collection efficiencies of various substances to be more adequately tested.

F. NON-METABOLIC GAS REMOVAL

Introduction

Because the instrument furnishes evidence of microbiological life through detection of metabolically evolved gas, it is essential that very little, preferably zero, radioactive gas from sources other than the collected microbes be admitted to the collector surface. However, the broth does exhibit a slight breakdown of its tagged constituents into nonmetabolic gases. Both space radiation and self contained beta radiation will contribute to this breakdown, the internal radioactivity being the dominant factor. Since neither source of radiation can be prevented from producing traces of gas, there exists the necessity of removing these unwanted products before the baffle is opened to admit incubation chamber gas to the collector. Removal of nonmetabolically evolved gases, then, must be accomplished after the broth ampule is broken but before the baffle is opened.

The method by which the gas will be removed was originally planned as a flushing process -- one in which a gas would be forced through the liquid broth by introducing it at the center of the string-chenille spool and allowing it to pass radially through the broth-saturated string. The present instrument, Gulliver III, has provisions for this type of flushing. Subsequent to these plans, experiments were performed to evaluate the effect of flushing techniques. These experiments led to another likely method of removing the nonmetabolic gases - simply allowing them to expand and diffuse out at the relatively low pressure of the Mars atmosphere.

The initial experimentation and design was based on estimates of the amount of flushing gas which would be necessary to purge the broth of tagged gases. RRI personnel felt that a volume of flushing gas about 10 times the volume of broth would be sufficient. Two methods of furnishing the flushing gas were investigated; the first was by chemical generation, while the second was by means of a small tank of compressed gas.

CO_2 As a Flushing Gas

Probably the most significant kind of gas to be removed is carbon dioxide. Flushing with "cold" CO_2 seemed the best choice because of the exchange between cold and hot molecules in solution. This exchange or dilution plus the effect of agitation was believed to be enough to purge the nonmetabolic hot gases from the incubation chamber, after a reasonable time for diffusion of the freed gas out through the string ports.

The objective of producing a feasibility demonstration model of the instrument at an early stage of the contract period required that some system of flushing be provided, and both methods of supply CO_2 were given simultaneous consideration.

The chemical generation source was studied in the laboratory by combining powdered carbonates and powdered acid, then initiating CO_2 evolution by adding water. This was thought to be the most feasible chemical method for the instrument since the reaction was controllable and could be initiated at will by smashing an ampule of water.

The mechanical method of supplying CO_2 was developed as a small tank with a limiting orifice which opens by actuation of a pyrotechnic bellows motor. This has proved quite satisfactory for earth conditions, though the tank and valve are not considered space-worthy. A device capable of containing gas under pressure during the space voyage and delivering its contents on demand has been designed, but not built.

Though these efforts were required as rather long lead time investigations, subsequent experimentation has shown that CO_2 is not as favorable as had been supposed. In flushing experiments described below, comparing CO_2 to N_2 and air as flushing agents, no particular advantage of CO_2 over the other gases was noted.

Also, it was discovered that a marked disadvantage resulted by using CO_2 -- the residual gas, though not radioactive, contributed to saturation of the barium hydroxide collector coated on the geiger tube window. For these reasons, air has been used in recent field tests, rather than CO_2.

Description of Flushing Experiments

There was no concrete data on the amount of flushing which would be required -- the specified amount suggested by RRI was merely an estimate. This aspect of the instrumentation problem needed empirical data on which to base the design of a flushing system. The best approach was judged to be simulation of actual expected conditions, so apparatus was assembled which enabled us to place a dummy incubation chamber in an environment such as that believed to exist on Mars.

The experimental method devised was a radioactive tracer technique. The problem was to find out the manner in which CO_2 is eliminated from the incubation chamber by natural means and by flushing. By using tagged $C^{14}O_2$, the presence of the gas was detectable by a geiger tube, and the count rate at any time was directly proportional to the amount of gas present in the incubation chamber.

Figure III-8 shows the components of the experimental apparatus. The vacuum desiccator can be pumped down to as low as about 1.5 inches of mercury absolute. Gases can be introduced into the desiccator to simulate Mars atmospheric conditions. The gas buret allows precise measurements of the flushing gas quantity. Radioactive CO_2 inside the dummy incubation chamber is sensed by a thin window geiger tube as in the Gulliver instrument; the coaxial lead is plugged into a scaler for readout.

The radioactive CO_2 to be flushed was put into solution in non-tagged broth and loaded into ampules. The activity of the CO_2 used for saturating the broth was 10 microcuries per ml. An experiment was performed to determine the solubility of CO_2 in the broth: approximately 0.5 ml CO_2/ml of broth. Each ampule contained about 1.5 ml of CO_2 in solution and about 15 microcuries of activity. For an experiment, one of these ampules was sealed inside the dummy chamber in the same manner as the broth ampule is placed in Gulliver, i.e., in a spool wrapped with greased string and chenille with only the string port open for diffusion of gas from the incubation chamber.

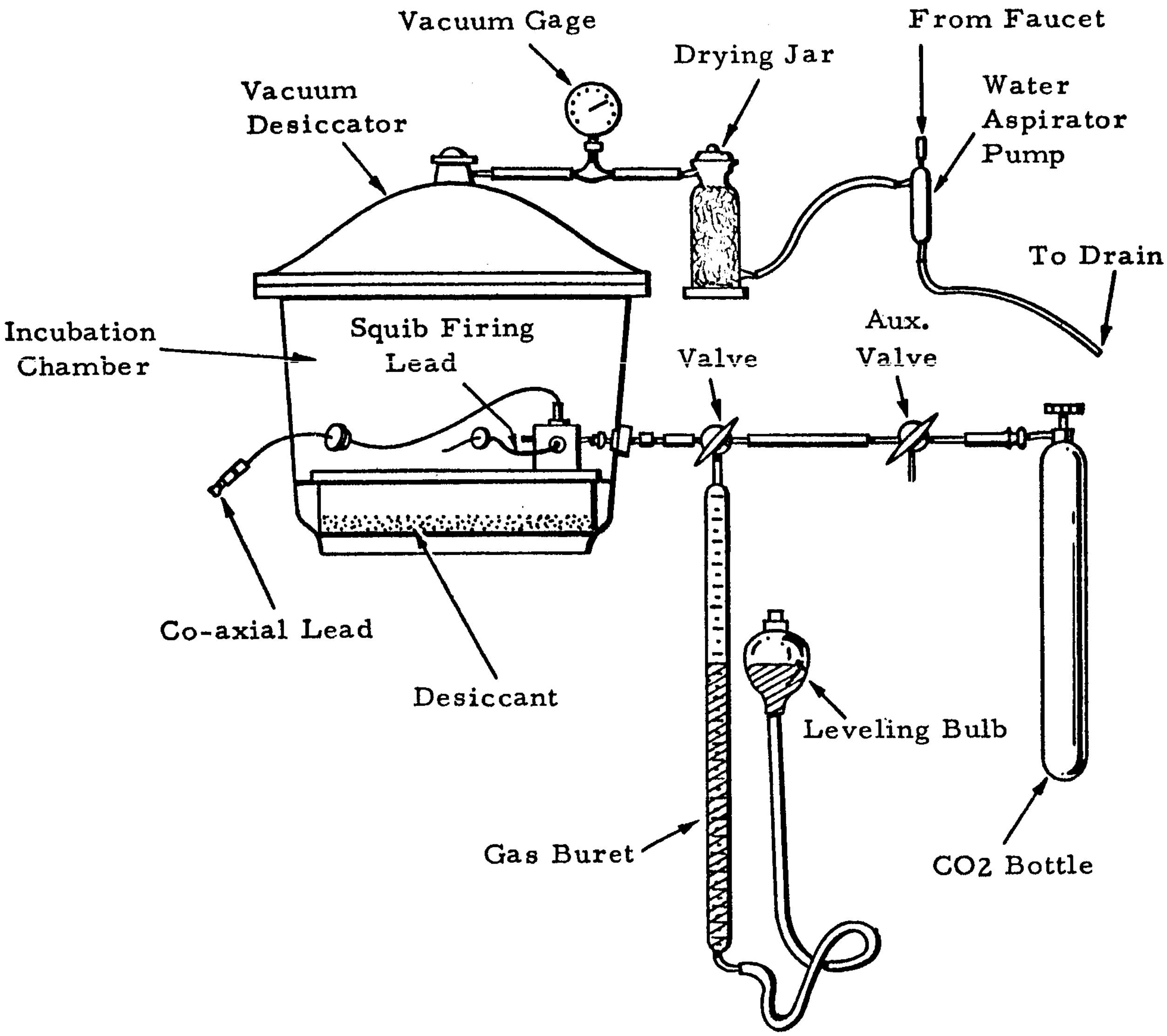


Figure III-8.

Environmental chamber for simulation of Mars atmospheric conditions — set up for measuring flushing gas flow through incubation chamber. The water aspirator pump maintains the desired pressure. Strong desiccants keep the chamber atmosphere dry. The environmental chamber can be modified readily for other experiments in the project.

Smashing the ampule by electrically firing a squib released the CO_2 saturated broth into the string, whereupon the behavior of the gas was observed by means of the counting rate.

In contrast to the Gulliver instrument, in which metabolic CO_2 is sealed in the chamber and absorbed on gas collector on the geiger tube face, this experiment allowed nonmetabolic CO_2 to escape and be driven out by flushing while a geiger tube without a gas collector detected the gas present at any time. Gulliver is a "static" measuring device while the apparatus used in these experiments was a "dynamic" system.

Though a complete description of all the experiments would be too lengthy here, a few of the most significant findings are included. A much more satisfactory knowledge of the behavior of nonmetabolic CO_2 in the Gulliver geometry and environment now exists than before these experiments were performed. These conclusions are summed up after the experimental findings for easy reference.

Non-Flushing, Earth Atmosphere Experiment

In order to get a "feel" for the apparatus and to obtain a basis for comparison, the first run was made outside of the desiccator and without flushing. Unexpected variables were encountered which required three runs to isolate the trouble. The effect of the fume hood air currents was so pronounced that changing the position of the hood door drastically altered the evolution of CO_2 from the dummy chamber. The air flowing past the string port apparently created a turbulence which caused the incubation chamber to be subjected to circulating air. This circulation swept out the CO_2 -- a direct relation between air velocity and count rate existed.

Flushing, Earth Atmosphere Experiment

With the dummy incubation chamber isolated from disturbing air currents, the ampule was smashed at "minute zero" and the count rate was recorded at each minute thereafter. No flushing was attempted until the downward trend was well established on the count rate vs. time curve. At 15 minutes, the gas buret containing 100 ml of CO_2 was emptied through the spool. The effect of this was disappointingly slight. At 45 minutes, the same flushing process was repeated with like effect. The count rate curve showing evolution of radioactive CO_2 from the incubation chamber is shown in Figure III-9.

Introduction of the flushing gas seemed merely to cause a brief stirring of the tagged CO_2, as shown by the small increases in count rate, but there was no appreciable drop from either flush. Though the amount and activity of the CO_2 released in this experiment were far in excess of what is likely to exist after the Mars voyage, the relative effect of flushing should be about the same. Due to the poor showing of the flushing technique, it was with considerable anticipation that we prepared the next experiment -- to break the ampule at the reduced pressure of the Mars atmosphere.

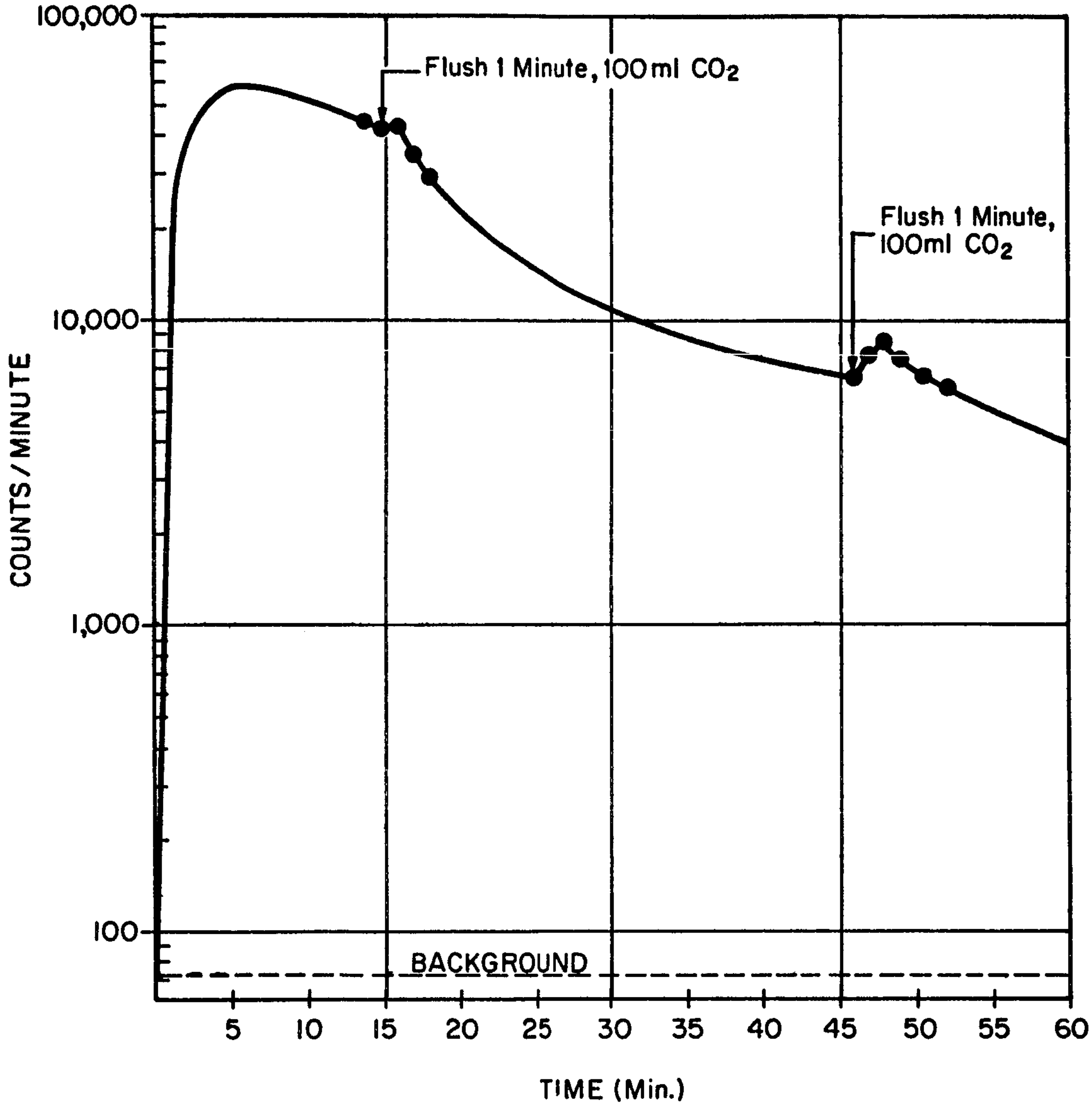


Figure III-9. Effect of flushing on nonmetabolic CO_2 in the incubation chamber at eartn atmospheric conditions. The amount of tagged gas present in the chamber at any time after the ampule was smashed is proportional to the count rate shown.

Non-Flushing, Mars Pressure Experiments

In a sense this run was a failure because the ampule had leaked most of its tagged CO_2 and the resulting curve was not comparable to the other experiments. But on the other hand, it is perhaps the most significant of all since it shows what is likely to occur with nonmetabolic CO_2 in quantities more closely akin to that expected in the actual Mars probe ampule. The curve of the results is shown in Figure III-10. With no influence other then reduced pressure of 1/10th earth atmosphere the count rate dropped into that of background in about 10 minutes.

In order to get more definite proof of the effect of pressure the next experiment was performed by smashing the ampule at 1 atmosphere and after the evolution rate slope was established, by pumping down to 1/10 atmosphere. The curves of absolute pressure and count rate showed a marked strong correlation.

The next run with a fully charged ampule broken at 1/10 atmosphere showed a rapid decline in count rate, but because of the high activity and amount of CO_2 in the "normal" ampule, the chamber was contaminated and the count rate never dropped to pre-experiment background. A check of the disassembled dummy chamber with a geiger tube survey meter showed no discernible activity remaining on the string or in droplets of free broth left in the chamber.

The evolution of tagged CO_2 under Mars pressure seemed very

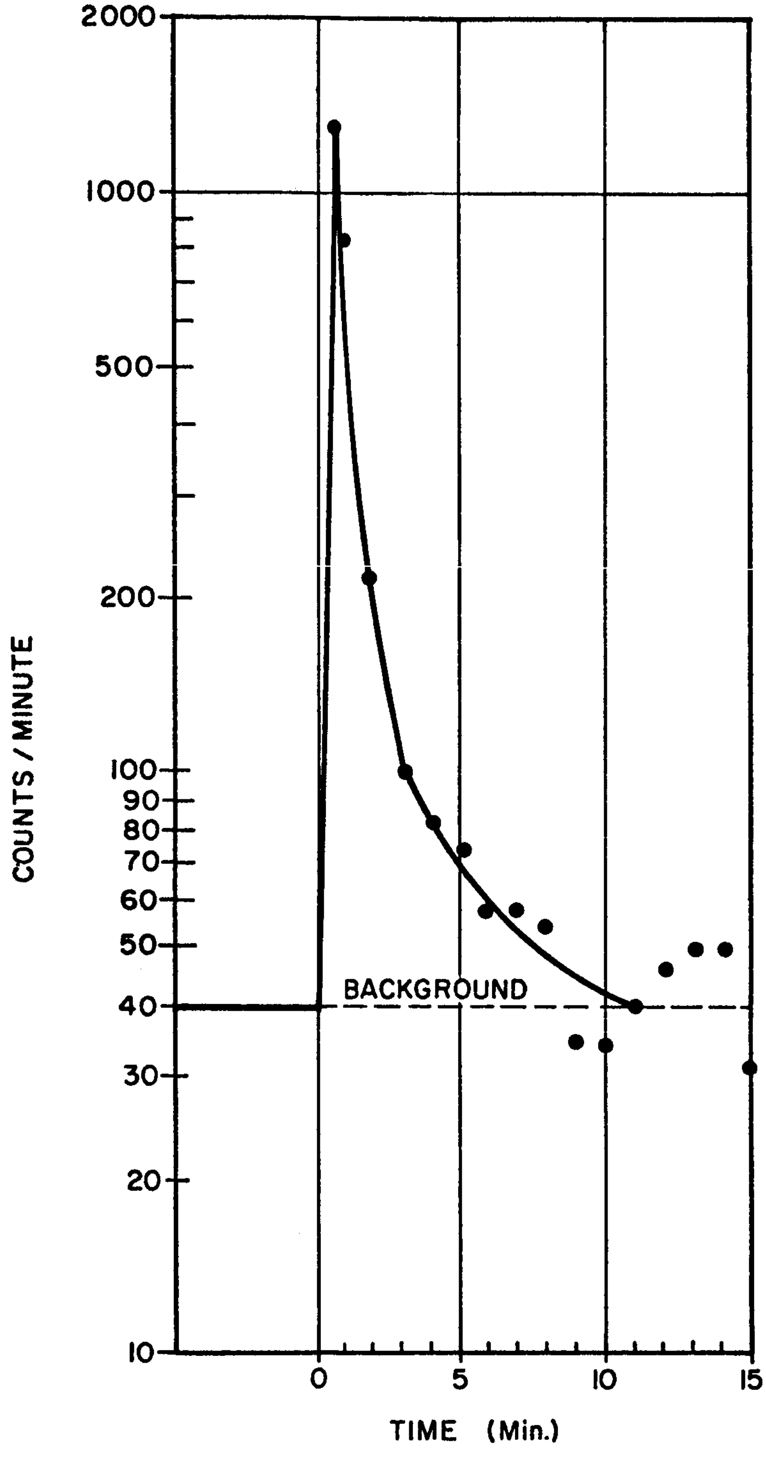


Figure III-10. Evolution of nonmetabolic CO_2 from the incubation chamber at Mars pressure. These results were from a lightly charged ampule of tagged CO_2. The amount of CO_2 present is proportional to the count rate.

satisfactory. However, one more uncertainty remained -- that being the effect of the CO_2 partial pressure on Mars upon the solubility of CO_2. The next run was made under conditions of Mars atmosphere composition and pressure.

Mars Atmosphere Pressure and Composition Experiments

A literature search was made to find out the best composition of gases with which to simulate the Mars atmosphere. The composition and pressure selected for the experiment were 98% nitrogen and 2% carbon dioxide at 3 inches Hg absolute.

The results of one of these runs are shown in Figure III-11. The Mars atmospheric composition in the desiccator did not affect the evolution of CO_2 from the incubation chamber enough to make any apparent difference when compared to the previous runs at the same pressure with air.

Miscellaneous Experimentation

After each run described above, various manipulations were performed in order to see what effects on the incubation chamber might ensue.

Flushing with nitrogen showed no difference in effect than with an equal amount of CO_2.

Varying the pressure in the desiccator always resulted in a proportional variation of count rate.

Certain parts of the incubation chamber always become contaminated during the tests with ampules of broth that had the full charge of $C^{14}O_2$ (about 15μc). This resulted in the geiger tube "background" counting rate after the

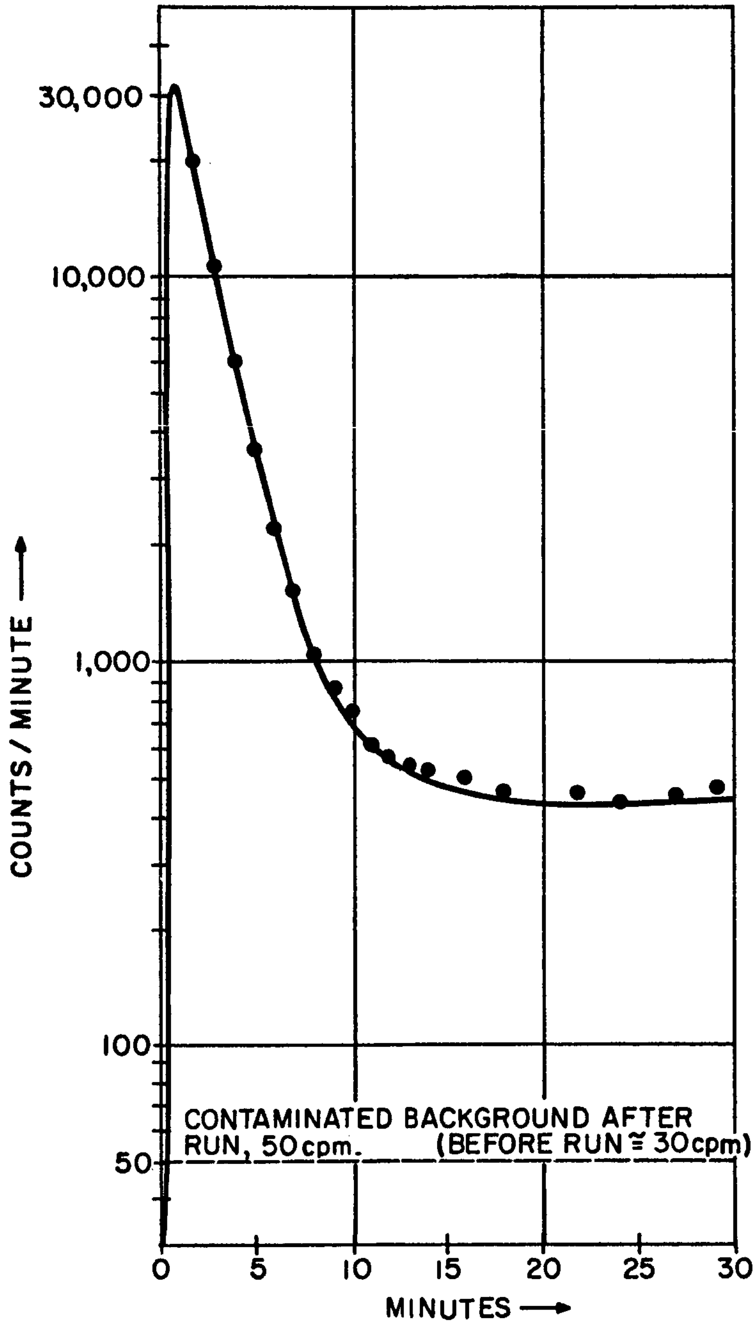


Figure III-11. Evolution of a fully charged ampule of non-metabolic CO_2 from the incubation chamber under Mars atmospheric composition and pressure. The amount of CO_2 present is proportional to the count rate. Note that the large amount of activity released in this experiment resulted in contamination of the chamber.

test being higher than at the start of the test before the ampule was broken. Admitting air through the incubation chamber to bring the desiccator back to atmospheric pressure (a thoroughly vigorous flush) brought the count rate down to the post-experiment background, but the count rate would then begin to climb to the level existing before "devacuumizing." This behavior was attributed to absorbed CO_2 and to flushed out gas reentering the incubation chamber from the desiccator. The broth-soaked string and chenille, when put to the window of a geiger tube survey meter, never showed activity after a run at low pressure.

Conclusions

There are two factors which affect the evolution of nonmetabolic CO_2 from the incubation chamber. The first is solubility of CO_2 in the broth; the second factor is diffusion out of the incubation chamber of CO_2 which has come out of solution. CO_2 partial pressure and temperature of the broth affect the solubility, while absolute pressure outside the chamber is the primary parameter affecting diffusion. If the ampule is broken at Mars pressure, diffusion out of the incubation chamber string port is the important factor; this is controlled by the absolute pressure.

Most of the benefit to be derived from flushing is in aiding the CO_2 to come out of solution, but the observed effect is slight at earth atmospheric pressure, and furthermore seems to be of no consequence if the ampule is broken at Mars pressure.

CO_2 comes out of solution readily under low pressure. Also, the suddenly released gas diffuses out of the incubation chamber readily at low pressure. It seems that nonmetabolic CO_2, when released on Mars, will diffuse out satisfactorily if the string ports are allowed to remain open for a short while, say about 10 minutes. The curve of Figure III-10 substantiates this conclusion.

Since contamination from the overly active gas used in all but one of this series of experiments prevented a return to pre-experiment background count rates, it is imperative that the results as shown in Figure III-10 be verified. This is the next step to be accomplished. If the curve of Figure III-10 is found to be a true representation, we will have evidence that flushing is unnecessary.

As an ultimate proof, the geiger tube window will be coated with barium hydroxide and be sealed from the incubation chamber for the period found best in the dynamic experiments. Then the baffle will be opened and the chamber sealed to allow any residual CO_2 to be absorbed on the geiger tube. If non-metabolic gas continues to evolve in quantities sufficient to cause an unacceptable sterile control level, the problem will have to receive more attention; if the amount of CO_2 left in the sealed off chamber is negligible then we can say that the problem is solved.

G. TEMPERATURE CONTROL

Tests were carried out on the heating system of Gulliver III to evaluate two areas of concern: (1) determine if the system would maintain the biologically preferred temperature range of 20°C to 30°C throughout the incubation chamber for terrestrial field testing; (2) establish the heating power requirement of the system as it now exists with little heat loss protection.

The tests were carried out in the following manner: Thermocouple junctions were placed in nine strategic locations in the Gulliver unit. The unit was placed in a temperature control box which was maintained in the temperature range of 10°F $\pm$ 2°F (- 12°C $\pm$ 1°C). With this arrangement four experiments were conducted.

1. To test temperature distribution, readings were taken for the nine junctions at three different stages of temperature regulation: at the lowest, the highest and a medium temperature. The results of this experiment, given in Figure III-12, show temperature regulation for most of the incubation area to be well within the desired range. They also show a very close correspondence of temperature differentials for each point, i.e., the change in temperature for one point being almost the same as that of every other point. To double check these overall temperature readings a close set of temperature versus time readings were taken for the thermostat junction (Figure III-13). The 5°F span indicated by these data is in good correlation with the heat distribution data.

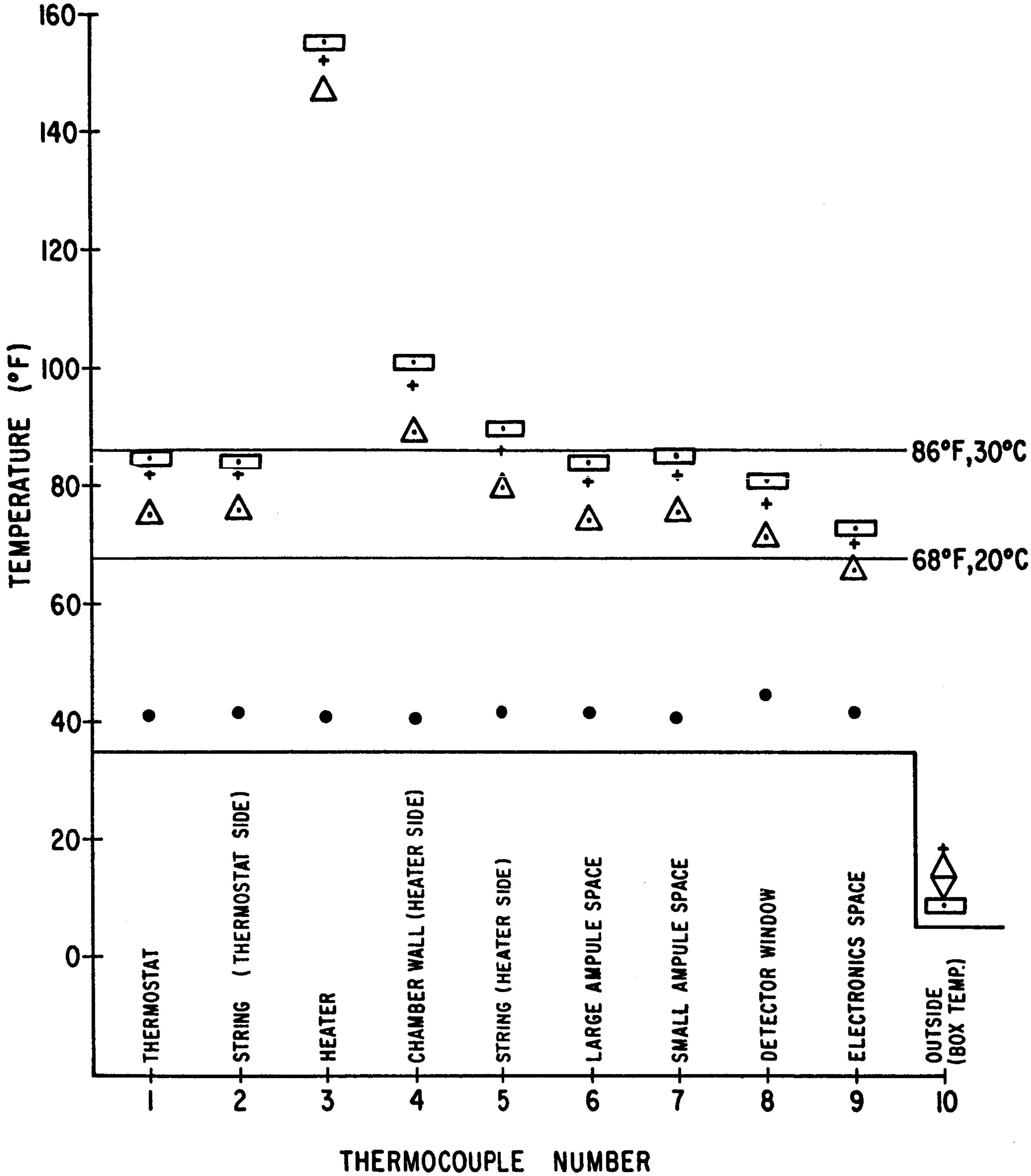


Figure III-12. Temperature distribution for various heater activities.

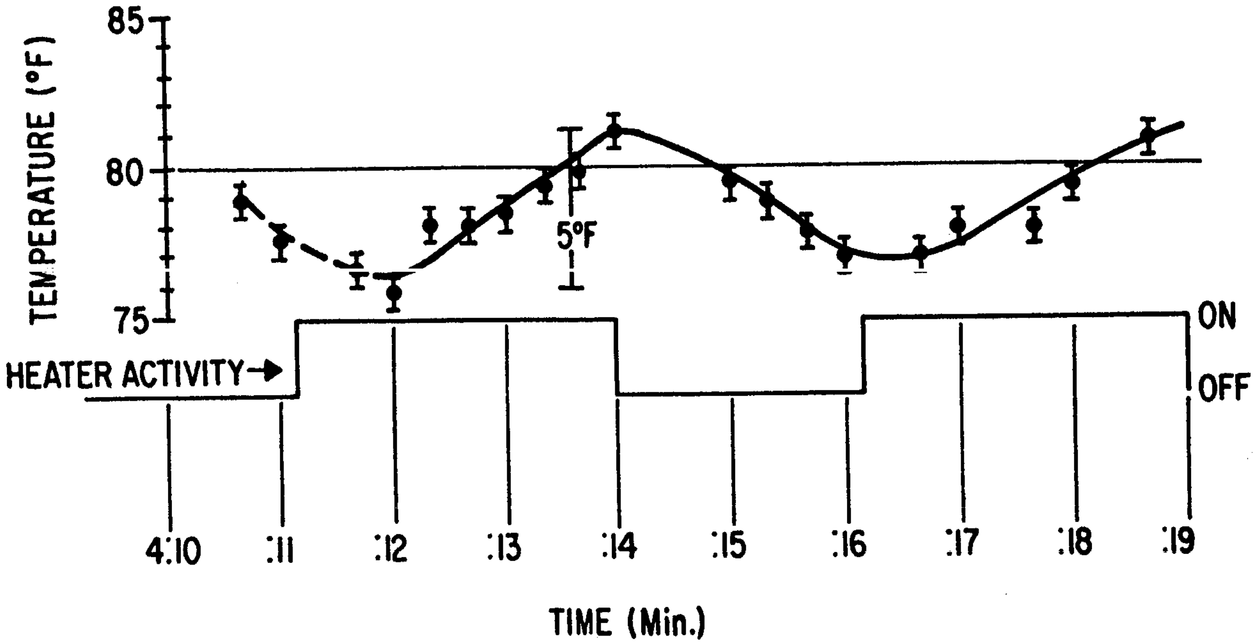


Figure III-13. Comparison of thermostat temperature and heater activity.

2. To check the range of temperatures of the area adjacent the heater for possible "hot spot," close temperature versus time readings were taken over one and a half cycles for the junction placed on the chamber wall across from the heater as in Figure III-14. These data provide interesting "phase lag" and temperature gradient information when compared to the thermostat temperature readings of Figure III-13. The danger of overheating is judged to be negligible on the basis of these data.

3. To provide power drain information during the initial heating up period, temperature versus time data were tabulated for the time between turning the heater on and the time it began to regulate. These data (Figure III-15) indicate a 35 minute constant power drain at 15 watts to raise the temperature 45°F, an energy equivalent of about .19 watt-hr/°F.

4. To determine heating efficiency after regulation begins, time versus heater activity data were taken. This is illustrated in Figure III-16 (Top). If "efficiency" is defined:

$$\text{Heating Efficiency} = \frac{\text{Time "Off"}}{\text{Total Time,}}$$

the value for this experiment is 38%.

On the basis of the last experiment certain modifications were recommended to provide thermal insulation of the incubation chamber from the base plate. Teflon screws were used to connect the unit to its supporting rods and the rods were covered with plastic sleeves. These modifications along with a more efficient alternating current power input resulted in

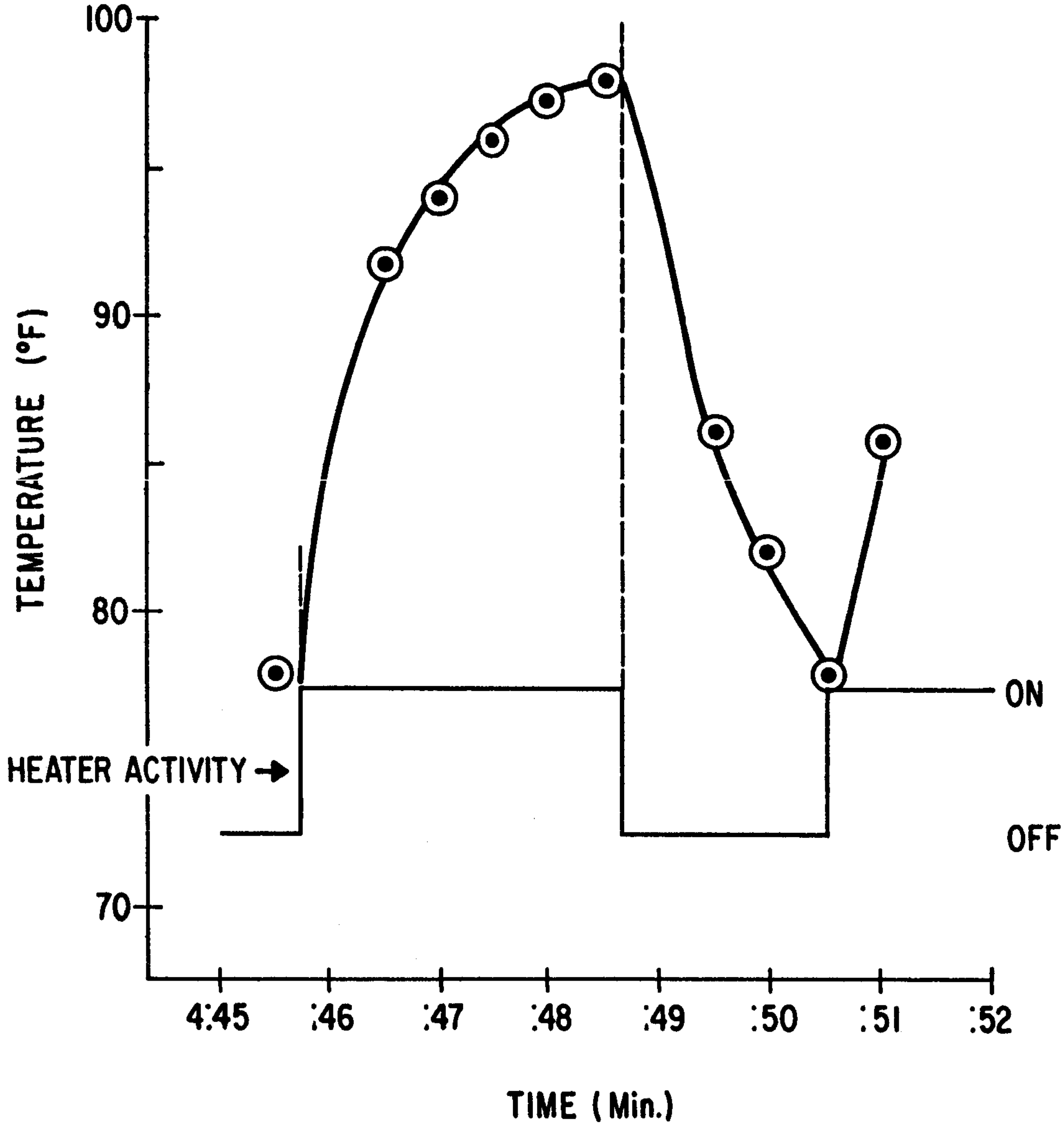


Figure III-14. Temperature vs. time at chamber wall adjacent to heater.

significant improvements in heating efficiency. During subsequent field tests where outdoor temperatures ranged from $25^{o}F$ to $35^{o}F$ heating efficiency was observed to be on the order of 90%, corresponding to an average power drain of 1.5 watts.

The system as shown in Figure III-15 follows: a 15 watt Electroflex Heater Type D (20 watts for the second unit) was attached to one side of the exterior incubation chamber wall with the Dow Corning Silastic 140 adhesive. This was covered by an aluminum canopy to provide an air pocket for insulation. A type MX-13 Termostat by Stemco was mounted to the opposite wall. This was rated at $77^{o} \pm 3^{o}F$. with a 2^{o}-$6^{o}F$ differential. The thermostat and heater were connected in series to a 28 volt AC supply.

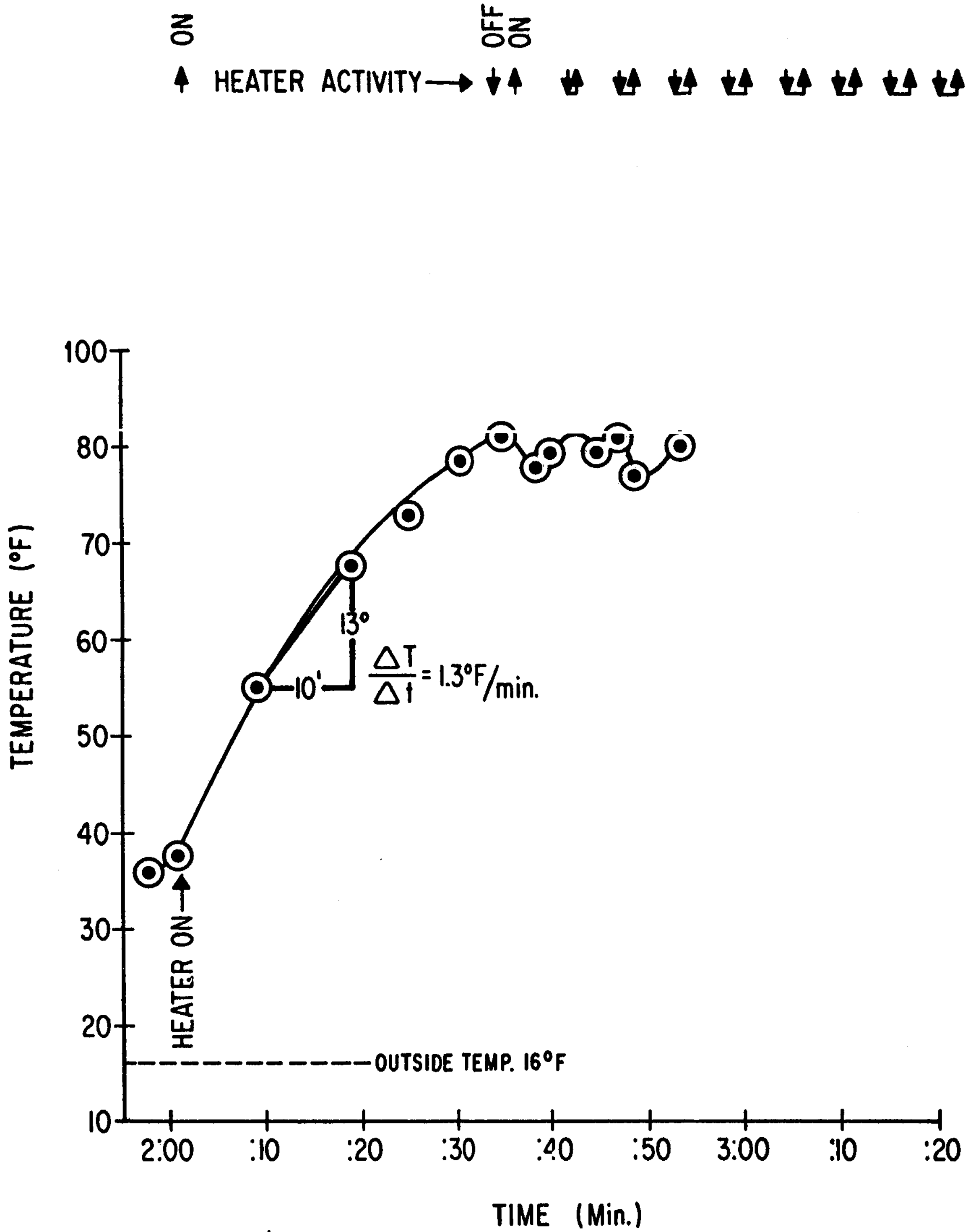


Figure 15 and 16. Thermostat temperature vs. time during initial warm-up to incubation temperature.

III. DETECTOR AND ELECTRONICS DEVELOPMENT

A. DETECTORS

1. Solid State Detectors

a. Circuits for Semiconductor Detectors

Amplifier Performance

The previous contract ended with a transistorized amplifier system that gave reasonably satisfactory performance in the laboratory. It was fabricated as a wired breadboard type model. Due to the high gain required for the overall system, it had a tendency toward oscillation. The oscillating condition was intermittent, and during field tests it often was required that the vacuum tube amplifier from the laboratory be used in its place.

The first efforts of the new program were to try to improve the amplifier stability and sensitivity. Experimental work was begun on new and different types of circuits. The most favorable of these circuits was selected and packaged on printed circuit boards. The preamplifier components were placed on 1-1/4 inch diameter wafers and then assembled into a tube of suitable diameter (See Figure III-17 and III-18). The semiconductor detector was mounted in one end of the preamplifier case with the sensitive area facing out. In actual use the preamplifier was mounted above the instrument body with the detector positioned above the gas collector at the top end of the throat from the incubation chamber.

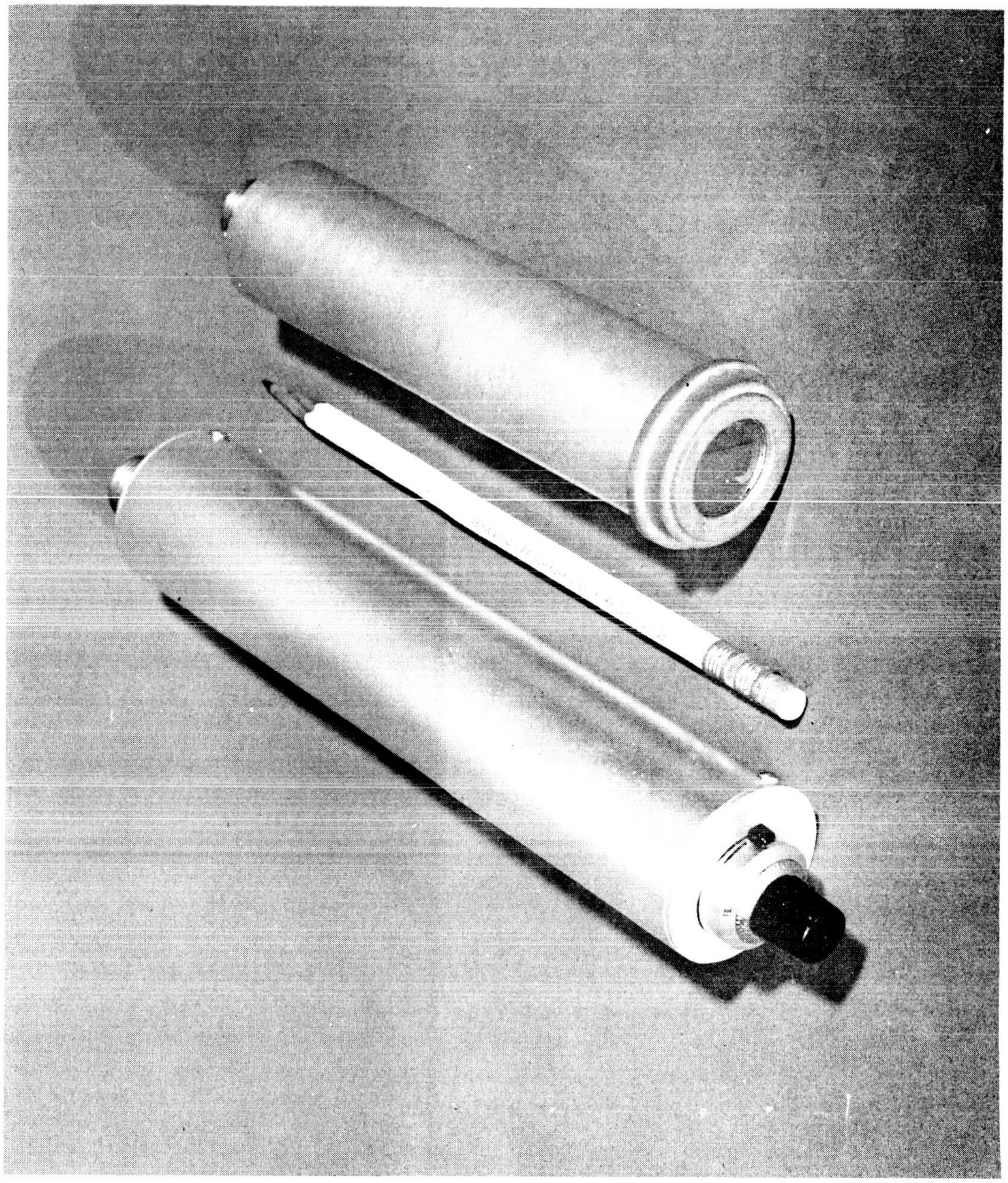


Figure III-17. Transistorized preamplifier and amplifier units designed for Gulliver III.

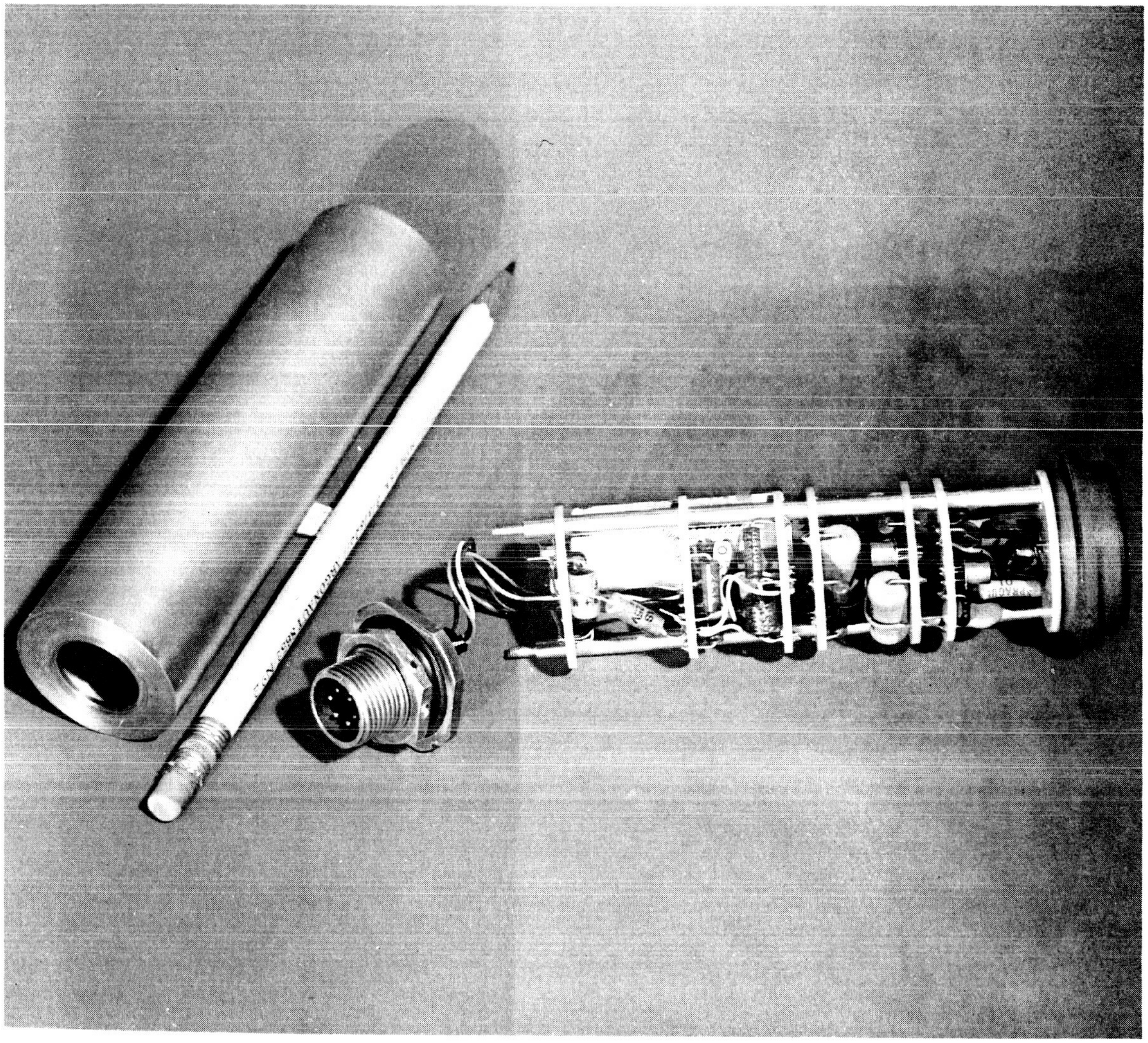


Figure III-18. Transistorized preamplifier removed from its housing.

The amplifier and discriminator are packaged in a separate case. The main reason for this separation is to reduce possibility of oscillation, but sufficient space for all components was not available in the preamplifier case that was on hand.

Amplifier Circuit Operation

The following is a brief description of circuit operation, see Figure III-19, the amplir schematic. Resistor R1 and capacitor C1 are used only when testing the amplifier with an external signal. An external pulse generator may be used to simulate the signals developed by the detector by inserting a low amplitude negative pulse at the input terminal. Resistors R3, R4, R22, and R23 provide proper bias voltage for the detector and capacitors C3 and C13 provide filtering for this bias voltage. It will be noticed that this circuit is voltage sensitive instead of the charge-sensitive type. The charge-sensitive circuit is the preferred type because the performance is not affected by changes in the input and detector capacity. The charge-sensitive feature was later added. The development of the voltage sensitive circuit was initially purused because it appeared to be stable and had no tendency to oscillate. Resistor R8 and Capacitor C5 decouple the supply voltage for the purpose of improving stability. The input signal is amplified by stages Q1 and Q2, then fed to stage Q3. The collector load for Q3 consists of a resistor, an inductor, and a capacitor. The purpose of this network is to provide shaping for the signal and in doing

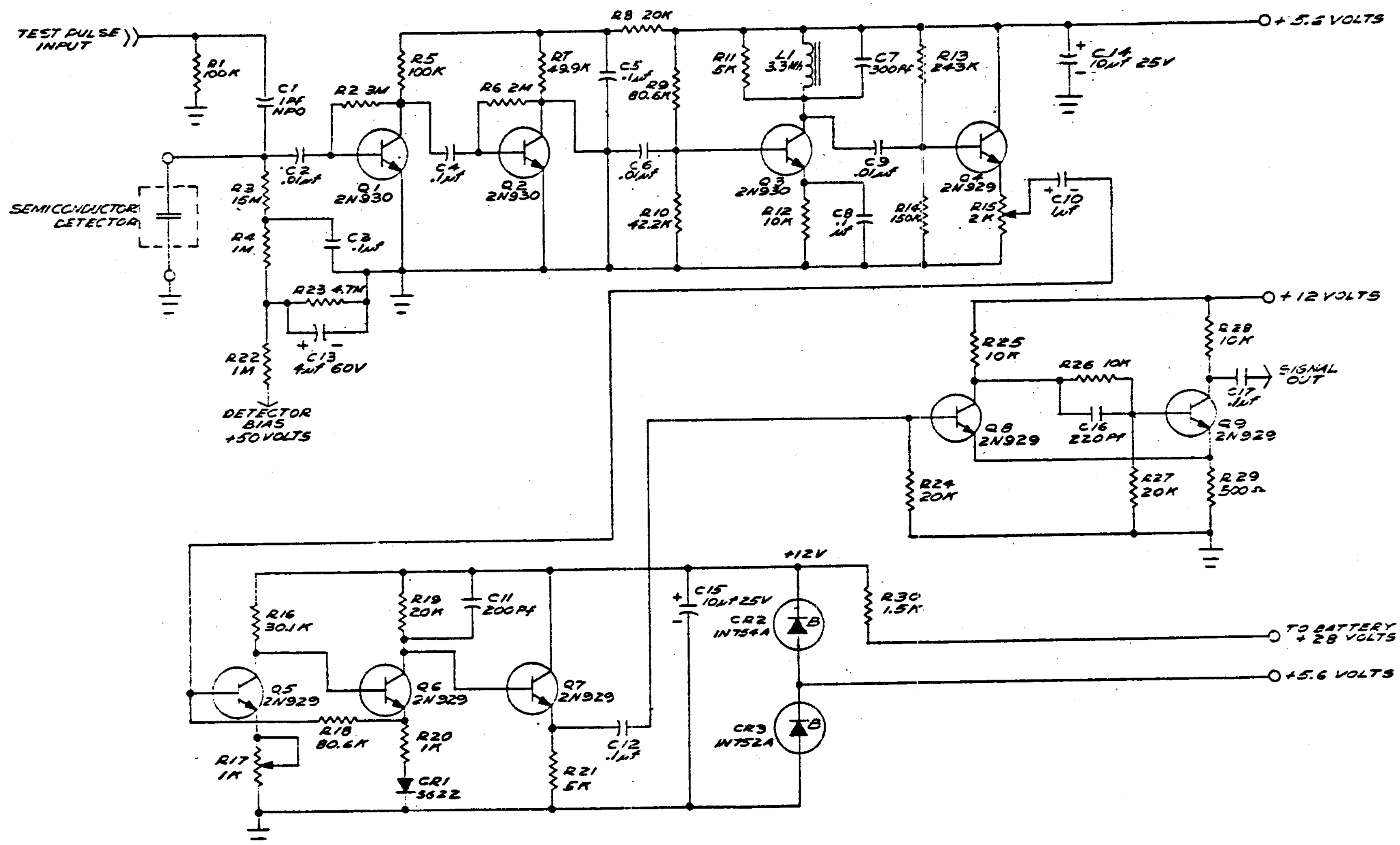


Figure III-19. Amplifier Circuit for Gulliver III.

so the low frequency noise signals are attenuated. The stage Q4 is an emitter follower which provides a low output impedance for the signal cable to the amplifier. Resistor R15 is a gain adjustment. Stages Q5 and Q6 form a termperature stable doublet. The diode CR1 corrects for changes in base to emitter voltage with temperature for the input transistor Q5. It should be noted that R17, the discriminator control, is in the emitter circuit of Q5 and when adjusted the temperature characteristics of Q5 are shifted. This is why good temperature stability is obtained between certain settings of this control. The capacitor C11 is used to reduce the high frequency noise which is outside of the passband in which we are interested. Transistor stage Q7 is used for impedance matching. Stages Q8 and Q9 form the Schmitt Trigger circuit. The trigger level can be changed by changing the value of R29, but the effective trigger point can also be changed by changing either the gain setting in the preamplifier or the discriminator control. Diodes CR2 and CR3 are silicon regulators. CR3 has a nominal voltage of 5.6 volts and CR2 has a nominal voltage of 6.8 volts so that the total is 12.4 volts. The 5.6 volt supply is used to power the preamplifier and the 12.4 volts is used for all other circuits.

Laboratory Tests

Several laboratory tests were made to determine the amplifier performance with different detectors, with battery voltage variations and with changes in ambient temperature.

The first test was to determine changes in performance as the detector bias and battery voltage is varied. The same discriminator setting was kept throughout the voltage test. The battery supply voltage was held to 21.73 volts during the bias test, and the bias voltage was held to 52.3 volts during the battery voltage test.

Detector Bias	One Minute Background Counts	One Minute Counts of C^{14} Source "A" Plus Background
52.3	25	3545
	28	3521
	29	
47.78	25	3250
	19	3126
43.11	20	2808
	12	2771
	18	2784
Battery Volts		
21.73	26	3545
	28	3521
	29	3521
24.00	50	4131
	60	4199
28.00	127	4732
	103	4802
	91	

A temperature test was made to determine performance at various ambient temperatures near the room ambient of + 25°C. Tests were performed at the four following temperatures: 0°C, + 25°C, +38°C, and + 55°C. The same discriminator setting was used for the test at all temperatures. A different C^{14} source was used during this test.

Temperature in °C	One Minute Background Counts	One Minute Counts of C^{14} Source "B" Plus Background
+ 55	119	6, 892
	160	6, 467
	117	6, 773
+ 38	94	11, 326
	116	11, 433
	104	
+ 25	71	13, 578
	92	13, 386
	82	
0	58	10, 974
	63	10, 889
	65	11, 002

A test was performed to determine how the count rate with a C^{14} source varies with different background (essentially noise) count rates. The data listed below show the results of this test. In performing this test the discriminator was adjusted to obtain the different background count rates. These were recorded along with the corresponding discriminator setting. A source was inserted and one minute counts were made at the various discriminator settings. In order to obtain the true number of counts produced by the C^{14} source, the background counts must be subtracted from the total C^{14} counts per minute at each setting.

One Minute Background Counts	One Minute Counts of C^{14} Source Plus Background	Net C^{14} Count Rate
13	1,979	1,966
26	2,734	2,708
67	3,608	3,541
154	4,577	4,423
350	5,890	5,540
925	7,466	6,541
1806	9,799	7,993

The conventional criterion for an amplifier for use with semiconductor detectors is its resolution as measured in kev, fwhm. This was discussed at length in the previous annual report.

The resolution of the amplifier designed and constructed for this project is 38 kev, fwhm with a 100 pf capacitor across the input. The resolution of a good commercial transistor amplifier that came on the market just recently is 53 kev, fwhm with identical input capacitance.

Further Improvements

A charge-sensitive circuit was developed which has proven to have sensitivity equal to that of the voltage sensitive circuit described above.

The advantage of a charge-sensitive preamplifier is that variations in the detector capacitance, caused by fluctuations in detector bias supply, ambient temperature, detector surface conditions, etc., do not affect the detector output signal amplitude as they do in a voltage sensitive configuration.

Current developments in the field of low-noise transistors were closely followed for possible application in this circuit. The possibility of using the recently developed low-noise field-effect transistors was investigated. This device has a lower noise figure and a higher input resistance than most transistors. The units presently available have a high input capacity with a resultant shunting effect that limits their usefulness to amplification of low frequency signals. Consequently, the investigation of field-effect transistors was discontinued as they could offer no improvement of noise figure over that of transistors already in use.

b. Results of Detector Tests

Application of Gas Collector to Detector Surface

Q In field and laboratory tests, the semiconductor detector surface was always protected by a thin mylar film to prevent radiocontamination. In view of the fact that in the actual device this film would be eliminated to attain maximum sensitivity, it was deemed necessary to determine the effect when the gas collector was applied directly to the detector surface.

The testing technique was to gather reference data with a standard C^{14} source and a 2 cm^2 solid state detector. While holding the geometry constant, successive layers of 1/4 mil mylar were placed between the source and detector and the count rate taken for each additional layer. This gave the effect of areal density of mylar absorbers on counting efficiency for the particular geometry, source and detector (1/4 mil uncoated mylar has an areal density close to 0.9 mg cm^{-2}).

The detector was then weighed, coated with $Ba(OH)_2$ and reweighed. The areal density of the collector was thus determined by weighing to be 2.1 mg cm^{-2}. The detector was then tested with the same source under noise background and geometrical conditions identical with the tests made prior to the coating but with no mylar absorber. The count rate was observed to correspond to that with two 1/4 mil mylar layer absorbers or 1.8 mg cm^{-2}. This value for the areal density, obtained by measuring C^{14} beta transmission rates through known mylar thicknesses and then through the applied coating, is very close to the weighed value of the coating, i.e., 2.1 mg cm^{-2}. Non-uniformity of the coating thickness as well as differences in the elemental constituents of mylar and the coating are two factors that may prevent a better correlation. These data are summarized below.

Absorber Thickness	Count Rate Before Coating Thru Indicated Thicknesses	Count Rate After Coating Detector Directly
0	62,272	
0.9	49,729	
1.8	38,579	38,568
2.7	30,495	
3.6	24,078	

The coating thus seems to have had no detrimental effect on the detector performance since its counting efficiency before and after coating differ only by the predicted attenuation of the coating itself. This attenuation effect emphasizes the need for optimizing gas collection efficiency and capacity against collector thickness.

It should be noted that this test was only to determine the effect of $Ba(OH)_2$ on the detector; therefore no effort was made to obtain a very thin coating. A thinner coating would cause less attenuation and thus provide somewhat greater sensitivity. Furthermore, the final choice of gas collector and adhesive has not yet been made. Once this combination has been determined, it will be coated on a detector and tested by the above method.

<u>Optimization of Detector Area</u>

The effect of semiconductor detector size on pulse height resolution and noise level has been fully discussed in previous reports. In essence, the noise level increases with the detector area. Extremely low noise detectors have been fabricated with an area of only several square millimeters, thus making use of this principle. In this program, however, medium to large size detectors are required because the metabolically evolved gas is collected on a coating applied to the detector face and consequently the gas collection time is a function of the detector area.

Since the detector radiation sensitivity decreases with area while the gas collection efficiency rises, some method had to be found to optimize the area, i.e., select a detector size that gives maximum response taking both factors into consideration.

Experimental attempts to determine the effect of collector area size on gas collection time did not give a clear answer, but it was determined that the dependence on area was something less than the first power of the area.

Conway[1] discusses a similar problem and states that the collection rate depends on the geometric mean of the surface areas of the gas evolving and collecting substances, i.e.,

$$G.\ M. = \sqrt[N]{\pi_i\, x_i^{f_i}}$$

$$\text{Collection rate} \propto \sqrt{A_1 A_2}$$

where

x variable of interest

f weighing factor

A_1 is the evolving surface area

A_2 is the collecting surface area

This, of course, results in the collection rate varying with the square root of the collector area when the evolving surface area is held constant, and ties in well with our experimental evidence that the dependence is less than a linear function of area.

To obtain quantitative information on the effect of detector area on counting efficiency, a collimated C^{14} source was prepared in order that

1. Conway, E. J., Microdiffusion Analysis and Volumetric Error, Crosby Lockwood and Son, London.

counting geometry variations would not obscure the investigation of the noise levels in the detectors.

Three detectors were used in this test:

(1) 0.5 cm^2 detector, resolution 12 kev, FWHM

(2) 3 cm^2 detector, resolution 30 kev, FWHM

(3) 4.5 cm^2 detector, resolution 39 kev, FWHM.

All three were very good detectors, i.e., they have relatively good resolution for their particular size. The discriminator level was reset for each detector to provide a noise background count rate of about 40 cpm and the collimated source then counted with each. The table-3 shows the results of these measurements.

Area (cm^2)	Resolution (FWHM)	Net cpm	$\sqrt{\text{Area}}$ x cpm
0.5	12 kev	320	226
3	30 kev	180	312
4.5	39 kev	100	212

Having obtained data on counting efficiency versus area, the optimum area can now be determined by weighting the count rates by the square roots of their respective areas. This is also shown in the Table 3. An unambiguous maximum is seen for the 3 cm^2 detector. Since only three detectors were tested, additional tests may shift the maximum point somewhat.

These data were taken with a low noise vacuum tube amplifier. As detector fabrication techniques improve, noise levels of the larger detectors could improve significantly, and the maximum would shift in that direction.

Comparison of Detectors

The following tests were performed in order to obtain quantitative data on the relative efficiencies of G-M tubes, solid state detectors and gas flow counters for the detection of C^{14}. More precisely the spectrum of interest was not that of C^{14} but a modified one due to the self-absorption of the gas collector. In order to obtain this condition, sources used in this test were $Ba(OH)_2$ samples, which had been exposed to $C^{14}O_2$.

Detectors used were:

1. 1.4 mg/cm^2 end window G-M tube
2. 3 cm^2 surface barrier solid state detector, resolution 30 kev, FWHM
3. Amperex side window (1/4 mil mylar) gas flow proportional counter.

Sources were much smaller than the detectors and were in close proximity to the detectors in order to minimize geometrical effects.

The results of the comparison tests are as follows:

	G-M cpm	Solid State cpm	Gas Flow cpm Inside	Gas Flow cpm Outside
Sample 1	7,700	5,200	16,000	8,100
Sample 2	20,000	16,000	45,000	29,000
Sample 3	30,000	22,000	66,000	40,000

c. Problems with Solid State Detectors

Sterilization Temperature Tests

The purpose of these tests was to determine the effect of sterilization and acceptance temperatures on semiconductor charged particle detectors, as outlined in JPL Specification 30257, dated 12 March 1962.

Test detector #1 was first tested at room temperature for stability, RMS noise, background count rate, and counting efficiency from a C^{14} reference source. It was then exposed to 130°C for 25 hours. Twelve hours after equilibration at room temperature, bias was applied to the detector. This voltage could only be applied very slowly as the detector noise would otherwise increase greatly. A period of eight hours was required before the operating bias of 50 volts was attained. After this condition was reached the RMS noise, background count rate, and counting efficiency were essentially the same as the pre-temperature measurements.

Test detector #1 was then tested at 145°C for 36 hours. Application of bias was begun twenty-four hours after restoration to room temperature. Again the bias increase had to be very slow; this time requiring two days to reach the operating level.

Post-temperature test measurements after the 145°C test revealed that the RMS noise level was higher and the counting efficiency decreased. An absolute determination of the change in the background count rate could not be determined due to a required tube replacement in the amplifier which caused a shift in the overall amplifier gain before a post-temperature test count could be taken. It is certain, however, that there was some increase in the background count rate since an RMS noise increase was observed.

Test detectors #2 and #3 were put through a pre-temperature test as described above and then subjected to 135°C for 24 hours. After return to room temperature, bias was applied slowly, but the RMS noise level decreased very little from its new value at no bias, to operating levels. Thirty volts bias were left applied for 24 hours with no improvement in the noise level.

In addition to the failure of the three above mentioned detectors to pass sterilization tests, experience with the stability of semiconductor detectors when operated within their inherent noise region raises doubts as to their suitability for such an application as Gulliver.

It was felt that a detector of greater long term stability was needed in addition to the more obvious requirement of passing the sterilization requirements. Later temperature tests (by a manufacturer) of a larger number of detectors than was tested at AMF revealed that if a detector once passed the 145°C test the probability was very high that it could take repeated cycling at that temperature. The manufacturer offered to provide further information when and if it is available.

This last information, coupled with awareness of the constant improvements in the state of the art of semiconductor detectors, lead the present investigators to believe they have a potential possibility for application in this program, but at the present their use would be unnecessarily risky. When the program was put into the 1964 Mariner B schedule it was imperative that the detector to be used be state-of-the-art.

2. GEIGER DETECTORS

a. Advantages

The advantages of a G-M tube insofar as this program is concerned is its long term stability and the simplicity of its associated electronics. This contrasts with the requirement of semiconductor detectors for an ultra low-noise amplifier of considerable gain when the detection of low energy particles is required. Furthermore, for the detection of low energy particles it is necessary to accept those noise pulses above the discriminator level, thus subjecting the "background" level to fluctuations in the inherent detector noise level.

The only disadvantage involving with the use of a G-M tube in this program is the mechanical integrity of the window, which must be necessarily thin for C^{14} detection. The present state of the art is capable of fabricating thin window G-M tubes capable of withstanding the required shock, vibration and temperature tests as well as the interplanetary vacuum.

b. <u>Anti-Coincidence Configuration</u>

<u>Geiger Detectors and Associated Circuitry</u>

There were a number of anticoincidence geiger systems considered--including those available from Amperex Electronic Corporation, Lionel Corporation, Eon Corporation, and others. None of the immediately available systems were completely satisfactory when weighed against our desires as to size, weight, power, etc. and against the required environmental specification for flight instrumentation. However, a very desirable geiger tube pair could be obtained from a very limited development program carried out by one of the geiger tube manufacturers, Amperex. To simulate the detector system that would evolve, an anticoincidence configuration with outside dimensions of about 2" diameter by 2" high was assembled using an Amperex 18515 beta detecting thin window geiger. This was "protected" by eleven Amperex 18550 gamma detecting geigers. These tubes can operate well at the same voltage (550 volts is being used). This configuration is shown in Figure III-5. The 18515 is a 1" O.D. by 0.67" long, thin end-window geiger. Limited anticoincidence protection is provided by the eleven 18550's (5/16" O.D. by 2" long) which are mounted around the periphery of the 18515 with their axes parallel to that of the 18515.

For the anticoincidence operation, the eleven "guard" tubes are wired in parallel and a pulse from any of these 18550 tubes coincident with a pulse from the 18515 beta tube blocks the pulse from the beta tube. Only those pulses from the beta tube appearing in anticoincidence (without a simultaneous pulse from the guard tubes) are permitted to be counted. The dead time of the guard tubes is about 250 microseconds and the beta tube dead time is about 75 microseconds. The dead time of the anticoincidence circuit is limited by the dead time of the detectors and not the circuits since the output pulse from this circuit to the counter or scaler is a standard 6 microsecond wide rectangular pulse.

A schematic of the circuits being used for the detectors is shown in Figure III-20.

The high voltage power supply for the geiger tubes is a Components Corporation Model 65-C, which is a potted unit. This supply operates from a 6 volt positive supply. The high voltage output is adjustable from 450 to 750 volts by changing the value of an external resistor. It provides 50 microamps at 550 volts. The regulation of the output voltage is $\pm$ 1% against input voltage from 5 to 7 volts and for the temperature range from 0°C to 50°C.

3. PROPORTIONAL COUNTERS AND IONIZATION CHAMBERS

An extensive literature search was made to determine if a detection system had already been devised that could be used in Gulliver to give increased sensitivity. Some of the publications searched were: Review of Scientific Instruments, Analytical Chemistry, Nucleonics, AEC publications,

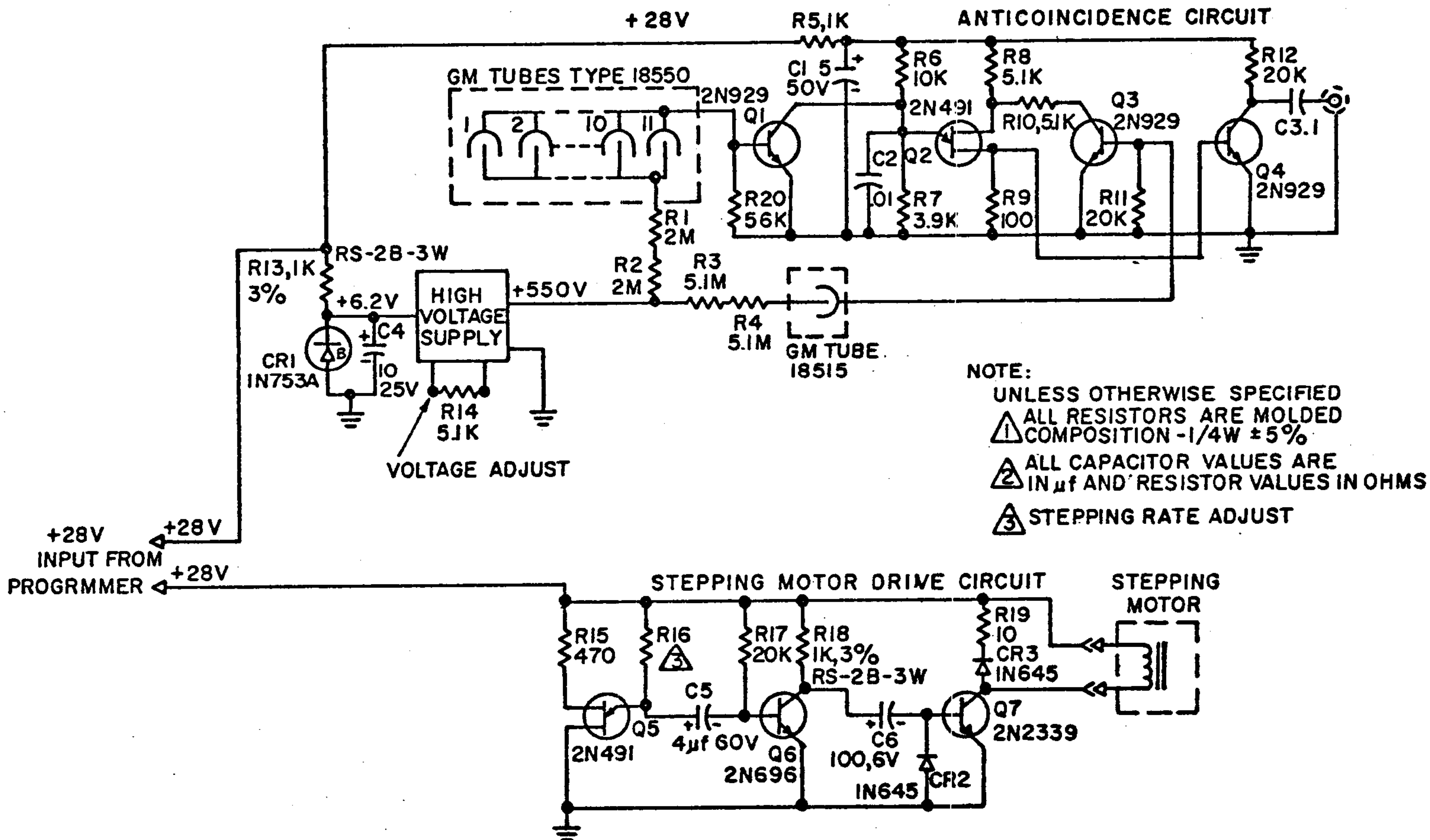


Figure III-20. Anticoincidence and Stepping Motor Drive Circuits for Gulliver III.

and Industrial and Engineering Chemistry. Most of the references were found in Nuclear Science Abstracts. None of the papers reviewed described a system that could be readily incorporated in the Gulliver instrument.

However, it appears as though a proportional counter could be designed that could operate directly above the incubation chamber, but separated by baffles. The $C^{14}O_2$ would diffuse up into the sensitive volume of the counter. for counting in the gas phase or on a gas getter. This opens the possibility for using H^3 tag in the broth.

A very recent preliminary test in the laboratorycomparing the sensitivity of a windowless gas flow detector to a thin window geiger indicates the windowless counter shows possibilities for greatly increasing sensitivity. Further testing must be done to determine the severity of such detrimental factors as of water vapor; other trace gases and gas pressurization.

B. FIELD TEST PROGRAMMER AND POWER SUPPLY

The support instrumentation used for demonstrating the instrument includes a few items that require a 115 VAC power supply. While it is anticipated that commercial power will be available at most test sites, it was decided that a portable power supply should be available for tests where such power is not available. Power requirements are small, being only that necessary to energize an all-transistor scaler, a programmer, and perhaps a power supply used to provide heat for temperature control of the incubation chamber.

A battery-operated power supply (Figure III-21) was built to fulfill

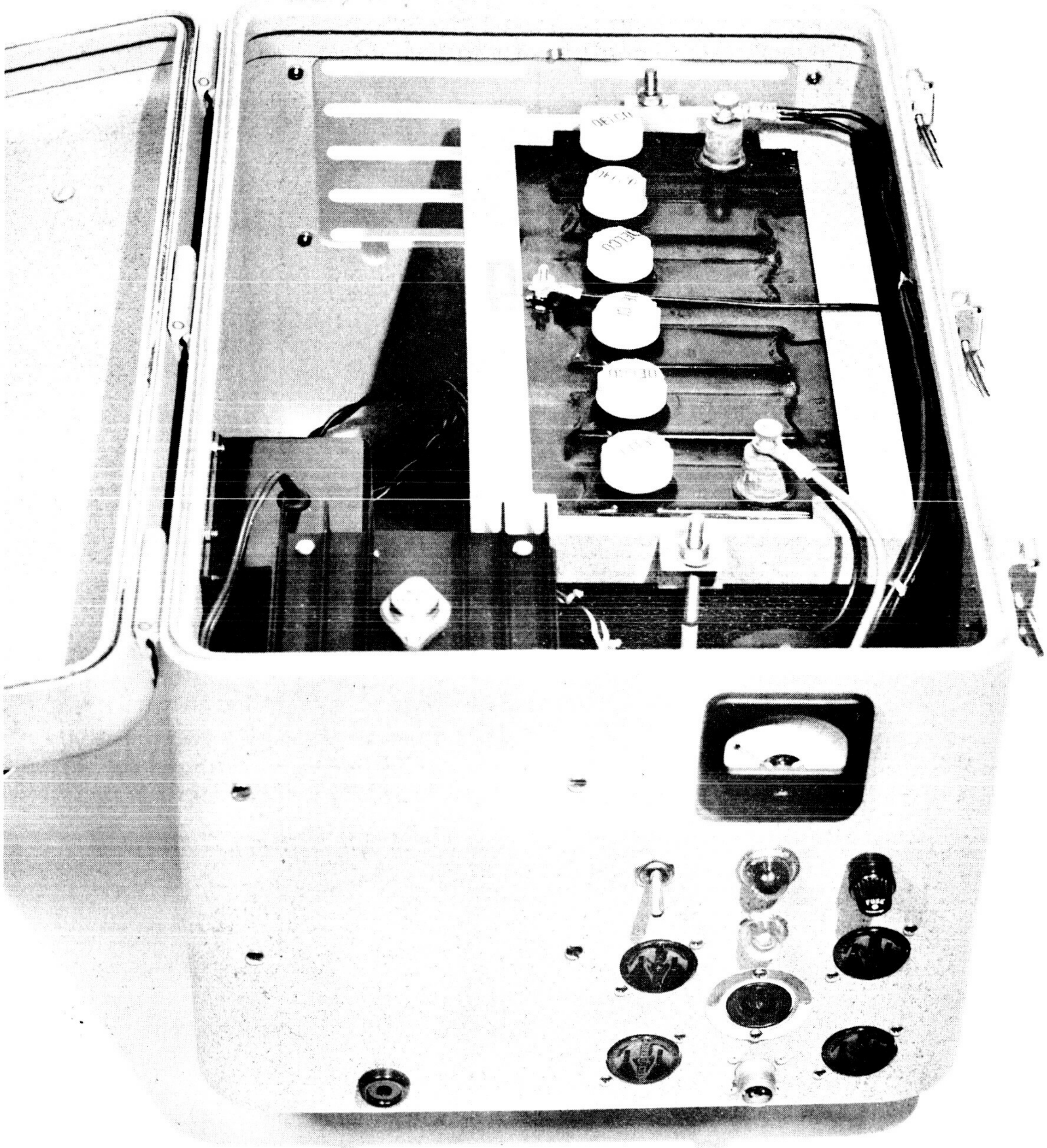


Figure III-21. Portable battery-operated 110 volt A.C. power supply.

these needs. A cabinet of suitable size was selected and inside were mounted a 12-volt sotrage battery (with a 6-volt tap installed), an inverter capable of supplying 240 watts of AC power continuously, a line filter, control switches, pilot lights, a fuze, output connectors, and a provision for charging the battery while in the cabinet. The cabinet was provided with two carrying handles and vents for the battery fumes.

Prior to the demonstration of the instrument on 19 April 1962, it was decided that an AC power-operated programmer should be available for testing the instrument with different time intervals being used for the functions. It was considered that such a programmer could be used as a standby in the event of failure of the small programmer previous. y developed. No effort was made to make the standby programmer small, since the principal objectives were flexibility and back-up. A nine-cam, electrically started timer was purchased and installed in a suitable case along with other auxillary relays that performed the same function as those miniature relays associated with the small programmer used for field tests. (See Figure III-22). The system was wired in such a manner that it could be directly substituted for the small programmer. This AC-operated programmer was used during the subsequent development of the life detection apparatus because of the ease with which the sequence of operations, the time for each operation, and the overall period of the cycle can be changed. The original effort for a flight-worthy programmer was discontinued when both

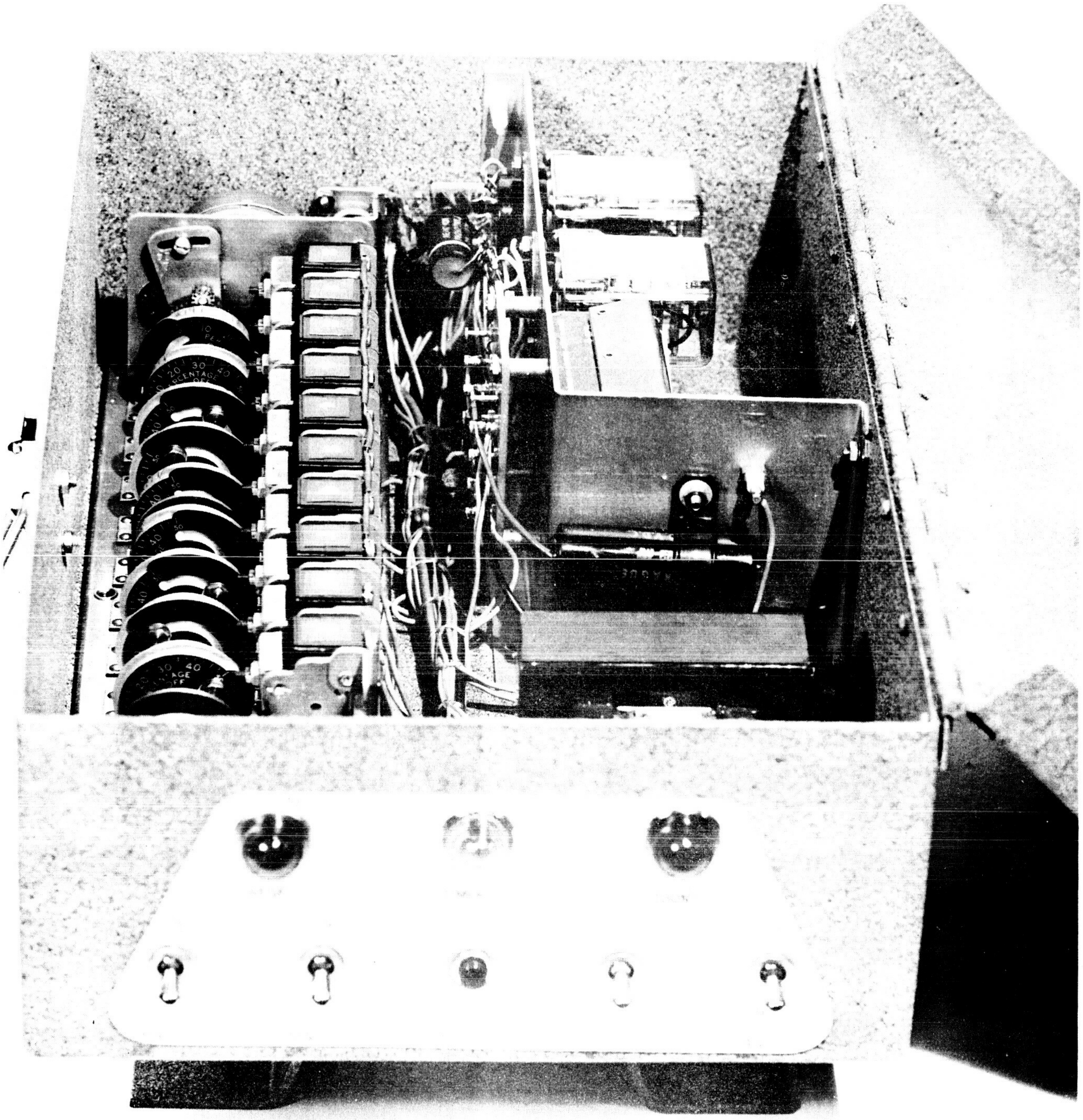


Figure III-22. Program timer for Gulliver III.

JPL and Goddard Space Flight Center informed AMF that the capsule timer would supply signals as needed.

C. LOGIC AND DATA HANDLING SYSTEM

1. Scaler and Logic System

Since it has been indicated that no scaler or logic system will be available on the Mariner B capsule, it is necessary that one be studied for this experiment.

A scaler is required to accumulate the pulses from the radiation detector and amplifier for the necessary counting periods. The experiment requires two identical chambers, either of which can serve as the biological control or the experiment. In order to provide this capability, it is necessary to provide logic circuitry capable of automatically sensing relative count rates from the two units. Since the background radiation intensity and spectral distribution is not known for the surface environment of Mars, it is necessary that the data handling system be able to sense relative increases in count rate above those count rates which are measured initially (before any biologically evolved gases contribut to the count rate). In order to do this, a system of data sotrage registers and controls is required. Refer to the block diagram in Figure III-23. The following is a description of the sequence of operations:

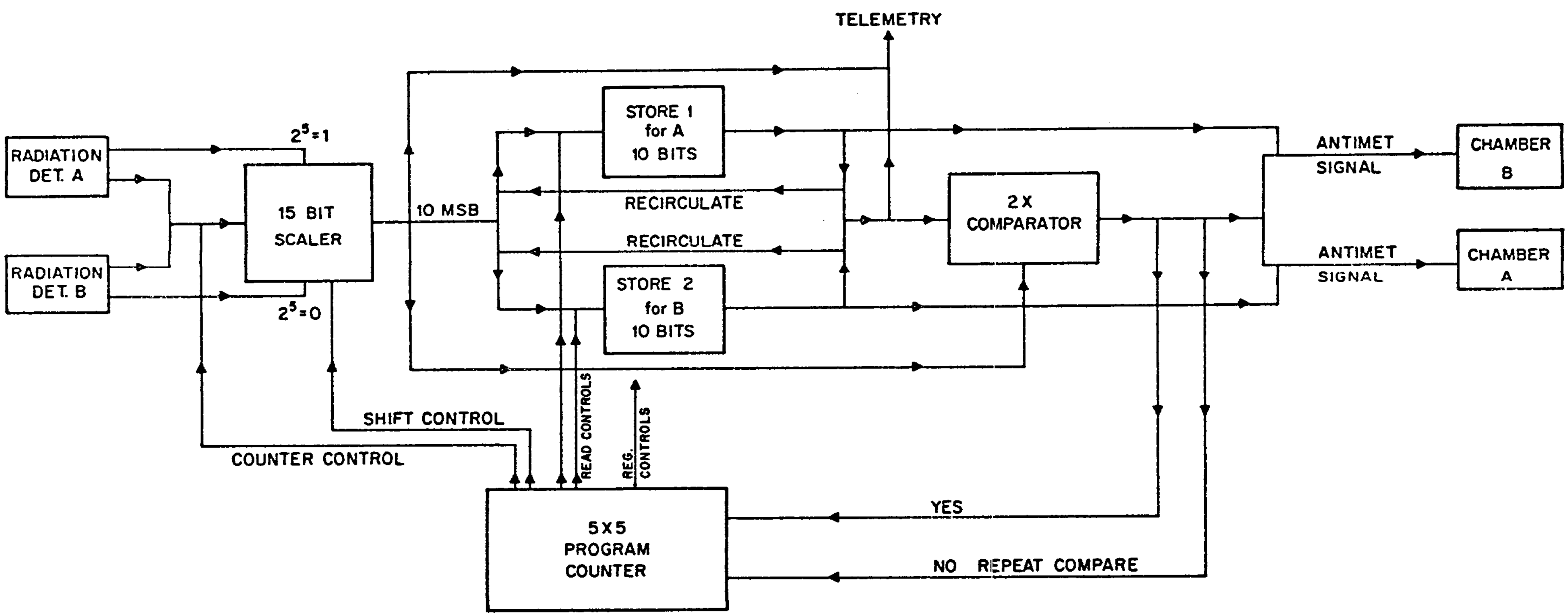


Figure III-23. Diagram of Data Storage Registers and Controls.

Operation

00	Reset all registers.
01	Count Probe "A"
02	Telemeter contents of Scaler - 10 most significant bits
03	Count from Probe "B"
04	Telemeter contents of Scaler - 10 most significant bits
05	Count from Probe "A"
06	Store "9" bits in store1, telemeter "10" bits
07	Count from Probe "B"
08	Store "9" bits in Store 2, telementer "10" bits
09	Count from Probe "A"
10	Compare contents of Scaler with Store 1 and telementer contents of scaler
11	Count from Probe "B"
12	Compare contents of Scaler with Store 2 and telementer contents of Sclaer
13	Count Probe "A" - Halt with night signal
14	Telemeter contents of Store 1
15	Time lost
16	Telemeter contents of Store 2
17	Time lost
18	Telemeter contents of Scaler

19 Count Probe "A"

20 Telemeter contents of Scaler

21 Count Probe "B"

22 Telemeter contents of Scaler

If the landing takes place in daylight, the logic would read as follows:

(a) Immediately upon landing the strings are ejected and retrieved. The signal recieved that the ports have been closed, meaning that the strings have been retrieved, shall initiate a signal resetting all registers and setting the control counter in operation.

(b) The first pulse received from the timer will start a count by Detector A into the scaler by a set time (arbitrarily 15 minutes). This will be followed by a second pulse which is used to step the program counter. This in turn shall be followed by a series of ten pulses which will be used to read out the contents of the scaler to the telemetery equipment. All single pulses will step the program counter.

(c) The third pulse will start a count from Detector B into the scaler by the set time, followed by a fourth pulse to step the program counter, followed by ten readout pulses which will be used to read out the contents of the scaler to the telemetry equipment.

The above account of operations will be uniform for the Day, Night or Partial Day operations. The next four operations (5, 6, 7, 8) will be to take counts alternately of Probes A and B and telemeter these back to earth; at the same time, the scaler contents will be stored in appropriate Stores using only the tem most significant bits. This will be read in using only nine bits into the stores -- effectively doubling the count. The 2^5 position in the scaler will be the bit used to identify the probe. A one in the 2^5 position in the scaler will signify Probe "A" and a zero will signify Probe "B". This will be the 2^1 position of the stores. The 2^0 position of the stores will not be deemed significant.

The next four operations (9, 10, 11, 12) will take counts alternately of Probes A and B and will be compared with their respective stores to find out which probe first exceeds twice its first stored count.

The scaler output will also be telemetered as the comparisons take place. The 2^5 position of the scalers will still identify the probe during telemetering.

Until the count from either probe exceeds twice its stored count, steps 9, 10, 11, and 12 will repeat indefinitely.

As soon as one of the probes has exceeded twice the stored count, it will cause the antimetabolite to be injected into the other chamber and the program counter will proceed to operation 19.

Operations 19 to 22 will be repeated indefinitely until a night signal is received. It will then halt until the night signal is removed.

Assuming that there has not been a doubling of the count from either chamber before a signal is received that night has arrived, the following describes the operations:

If the night signal is received during steps 9 through 12, or or more comparisons will take place. However, it is possible that many comparisons will have taken place before the night signal arrives; if such is the case, the loop (9, 10, 11, 12) will be broken at step 12 and a count will be taken on step 13. This will cause a halt after step 13 is performed before proceeding to step 14. The stores will contain 2X count A and 2X count B, with the scaler containing a count A. However, as this loop is being performed the contents of the scaler counts are being telemetered.

Upon removal of the night signal steps 14 through 22 will be performed and the loop 19 through 22 will have been entered, and repeat indefinitely.

If a night signal is received during steps 19 or 21, they will be performed and the program counter stepped to 20 or 22 and stopped until the night signal is removed.

Component requirements for a low power consumption system to perform the described logical functions would be 282 transistors, 600 diodes, 560 resistors, and 420 capacitors. The total weight would be approximately 2.3 pounds. There has not been sufficient time available to calculate accurately power requirements. A rough calculation has been made and requirements could be in the range of 90 to 180 milliwatts total. These requirements are based on using four transistors per flip-flop.

It is estimated that component requirements for this same system using only two transistors per flip-flop would result in a 33 percent saving in space and weight. Power requirements could increase to between 125 and 250 milliwatts.

Other scaler and logic systems have been conceived and studied which do not provide the versatility of the system described above. The luxuries of system versatility will depend, naturally, on availability of weight and power.

2. <u>Telemetry System</u>

It is desired to design a system whereby the outputs of two Geiger counter tubes and two temperature sensors are periodically sampled and coded in such a way that they may be read out to a transmitter capable of sending information at a one bit per second rate. The two Geiger counters involved may be considered to have firing rates anywhere between one pulse per second and 10,000 pulses per second. The experiment involved employs the Geiger tubes to measure radiation produced by the growth of bacteria. The rate of counting measures the rate of bacteria growth. Since power is at a premium and the transmitter itself consumes a large amount of power when turned on, the greatest magnitude power saving can be realized by reducing sampling rate and information data readout to a minimum, thereby reducing the transmitting time. Temperature sensors are included to monitor one of the most important variables, and it is necessary to convert the analog temperature measurement to a digital number for transmission concurrent with that of the data readout.

Tentatively, in order to conserve transmitter time, it has been decided to alternately sample each temperature sensor so that, if any data sampling occurs once every 15 minutes, each temperature sensor will be read every half hour.

A further refinement may be incorporated in order to reduce the number of data transmissions. The approach herein outlined may be essentially described as the use of logarithmic sampling intervals. In essence, because it is anticipated that the largest change of counting rates will occur during the first half of the duration of the experiment, it appears that the greatest sampling frequency is required at the very beginning. For an experiment lasting say 16 hours and only 6 sample readings possible, the most desirable approach would be to make the first sampling after 15 minutes, the second after 1/2 hours, and the third after an hour, etc. What has been utilized might be described as a logarithm to the base 2 time base.

<u>Timing Diagram</u>

The presently-planned telemetering approach will use a 14.5 minute data accumulation time, a 40 second readout time, and a dead period varying from zero to approximately 8 hours. See Figure III-24. Forty bits of information will be required to provide all the necessary data. The first 4 bits are a 1010 sync code. The next 16 bits are allocated to providing the significant bits of accumulated counts of Geiger Tube No. 1. The succeeding 13 bits for Geiger Tube No. 2 and the remaining 7 bits for the analog digital conversion of temperatue information.

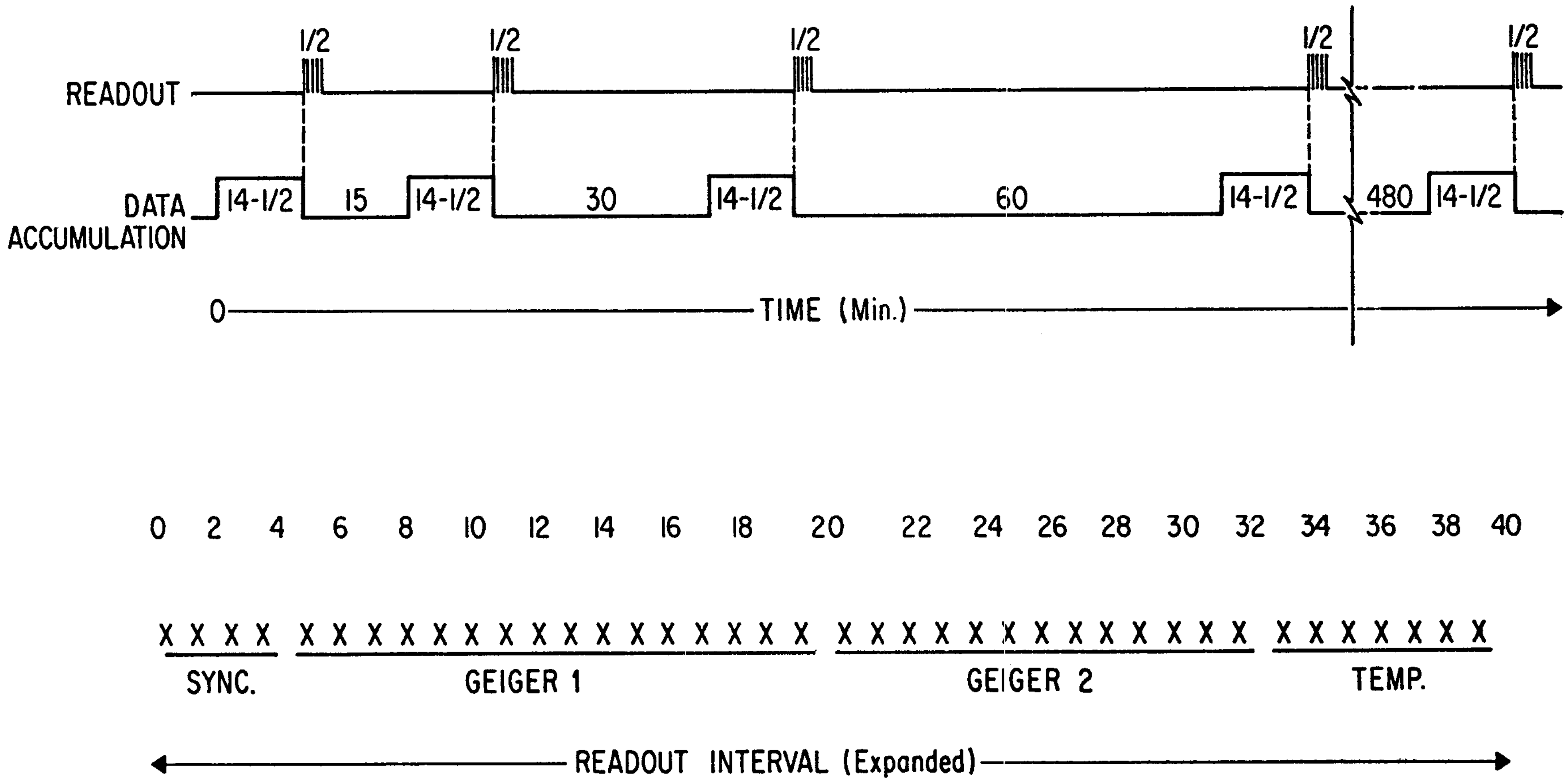


Figure III-24. Timing Diagram for Gulliver III Telemetry System.

Circuitry

The enclosed logic block diagram (Figure III-25) shows the method to be employed in data accumulation and telemetry readout. A one-cycle per second master clock is scaled down by 940 to 1. The first 900 counts are employed to allow for accumulation of data in the Geiger channels. The last 40 counts provide discreet sampling pulses for information readout. Each group of 940 pulses is counted in a scale of 64 each so that the transmitter is provided with information according to a base two logarithmic expansion. This is achieved by sensing the first, second, fourth, eighth, sixteenth and thirty-second group of 940 clock pulses. Note that in the Geiger channel, not all bits are reproduced as data. In each case, 7 bits are ignored. This is the equivalent of dividing the count rate of each counter by 128. If the minimum count rate is one count per second and a 15-minute time is allowed for data accumulation, the minimum number of counts from the Geiger tube would be then 1 x 60 x 16 or 900 counts. The resulting readout to the transmitter would then be approximately 8 counts permitting low count measurements with an accuracy of about 10%. This approach represnts the optimum compromise between transmitting time, low count accuracy, and statistical deviation of a random source.

The two temperature sensors involved will probably be resistive and as such an analog to digital converter must be used to provide a train of pulses to represent the sensor resistance. The circuitry shown provides

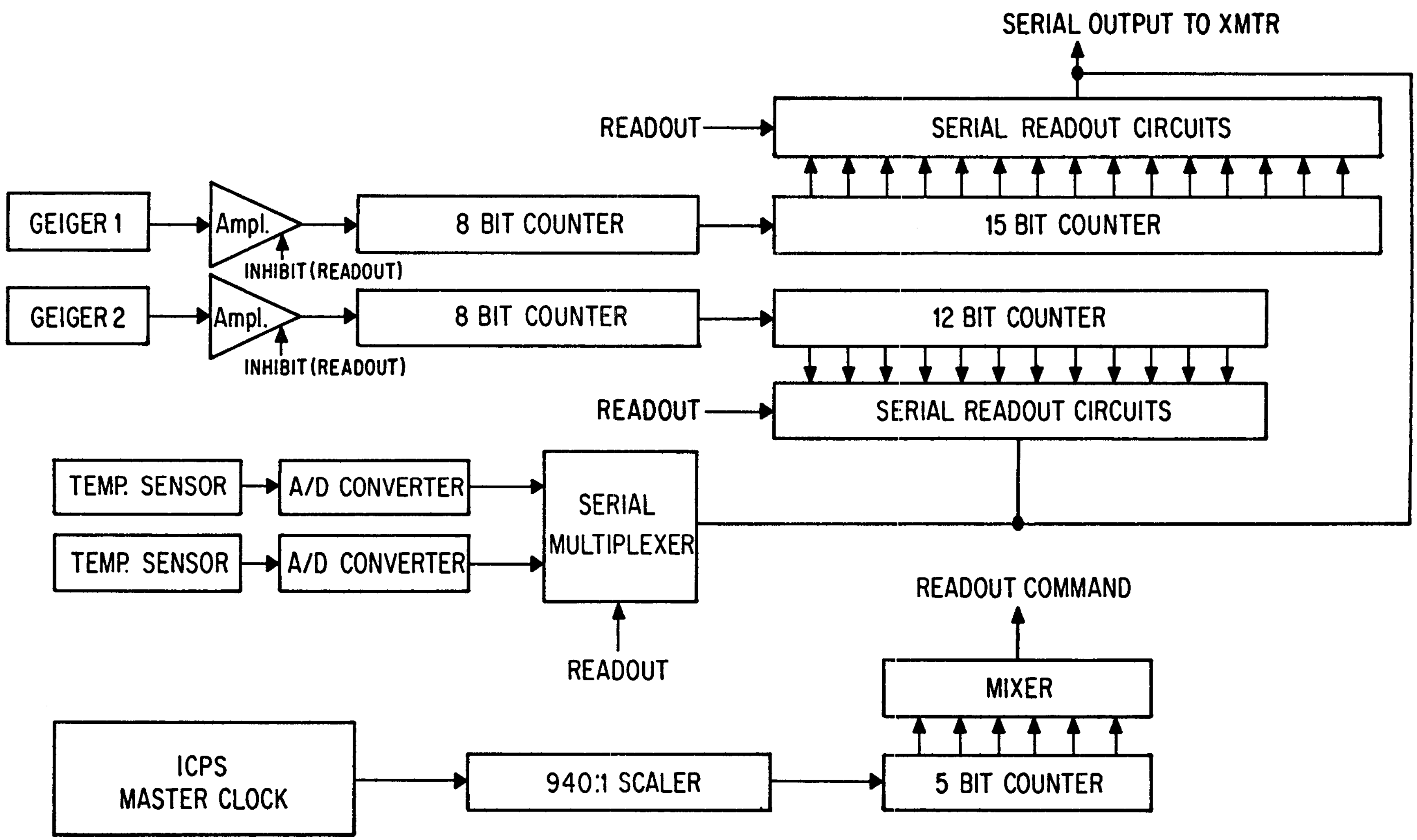


Figure III=25. Logic Blcok Diagram for Data Accumulation and Telemetry Readout for Gulliver III.

ultimate readout of each sensor. It might even be possible to use one A/D converter and multiplex the two sensors at the sources.

Power Consumption

It is estimated that two Geiger amplifiers will consume a total of 5 milliwatts. The 2 A/D converters total 100 mw.; the master clock 8 mw. There are 58 NORS using a total of58 mw., 67 flip-flops using 6 mw., and one delay multi using one mw. The total dissipation computes to a little less than 1/4 watt with power supply efficiency and temperature sensors extra.

IV. FIELD TESTS

Many field tests were performed in this phase of the program. These are summarized below. Many additional tests of particular components of the system were performed in the AMF Laboratories in the design phase of this effort.

Date	Sample Taken	Incubation	Equipment	Remarks
4/3/62	Sailing Marina	In AMF Lab	Gulliver II Solid State Detector, vacuum tube amplifier.	Slow response to 4000 cpm after 16 hours. Collector saturated.
4/9/62	Sailing Marina	Outside AMF	Solid State Detector, vacuum tube, amplifier.	5000 cpm after 12 hours, 30,000 after 20 hours.
4/12/62	Sailing Marina	In AMF Lab	Solid State Detector, vacuum tube amplifier.	700 cpm after 6 hours.
4/19/62	Sailing Marina	Sailing Marina	Solid State Detector, Transistor Amplifier	1000 cpm after 10 hours, 5000 cpm after 20 hours.
10/25/62	Ball Field	In AMF Lab	One Gulliver III Instrument	High Sterile level -- 6000 cpm after 1 hour, High response 15,000 cpm after 12 hours, 200,000 cpm after 24 hours, no flushing.

10/30/62	Ball Field	"	"	High Sterile level and high response, 2700 cpm after 1 hours, 60,000 cpm after 24 hours, no flushing.
11/2/62	None	"	"	Sterile Control, Broth auto-claved, 11,000 cpm after 1 hour, 11,000 cpm after 10 hours, flushed with 60 cc air.
11/7/62	None	"	"	Sterile Control, 780 cpm after 1 hours, 3200 cpm after 17 hours, flushed with 10 cc air.
11/16/62	Ball Field	"	"	Broth sterilized separately, unit assembled aseptically, 235 cpm after 1 hour, 18,000 cpm after 24 hours, flushed with 100 cc air.
11/21/62	Ball Field	Ball Field AMF Lab	Two Gulliver III's, one as a sterile control	Both units about same for 16 hours, sample unit 12,000 after 28 hours, sterile control unit 1500 after 28 hrs. both flushed with 100 cc air.
11/27/62	Ball Field	Ball Field, AMF Lab	Two Mark III's, one as a sterile	Sample 450 cpm after 4 hours, sterile control 225 gm after 4 hrs. sample 100,000 cpm after 24 hrs. sterile control 600 cpm after 24 hrs. both flushed with 100 cc air.
1/9/63	Ball Field	AMF Lab	Two Mark III's fired for samples	Operated upside down, both units failed due to electrical trouble in programmer. Both flushed with 400 cc air.
1/21/63	Ball Field	AMF Lab	Two Mark III's fired for samples	Operated upside down, little sample, mostly soot collected from sheet of ice. Unit 1-374 cpm from 2 1/2 hrs, unit 2 - 197 cpm after 2 1/2 hrs. Anti-

				Metabolite fired in Unit 2. Unit 2 - 600 cpm from 12 to 28 hours. Unit 1 - 8,000 cpm after 30 hours. Both flushed with 400 cc air.
1/29/63	Ball Field	AMF Lab	Two Mark III's fired for sample	Units operated upside down, Both failed due to excessive grease on lines which caused broth drippage.
2/7/63	Ball Field	AMF Lab	Two Mark III's fired for sample	Unit 1 - 2000 cpm after 2 hrs. 16,000 cpm after 24 hours. Unit 2 - anit-metabolite fired after 2 hrs, 1200 cpm after 24 hrs. Both flushed with 400 cc air.

The tests of October 25, 30, November 1 and 7, pointed up the high sterile control level from the broth. Resources Research reduced this level by changes in the concentrations of the tagged compound, by closer control of the autoclaving, by autoclaving broth and instrument separately and assembling aseptically. The results of the investigation to remove non-metabolite gas as described in the section IIF , as yet has not been incorporated into the testing procedure.

The tests of January 20, 29 point up the need for careful study of sample collection techniques with particular attention to the amount of grease on the sample collecting string.

There have been occasions when the gas collector was found to be saturated. As of now it is not clear how or when this occurs. An air port has been left open in the chamber to allow oxygen and atmospheric CO_2 into the chamber on the theory that both may become exhausted from air initially in the chamber before the collected microorganisms could really start multiplying. However, these open ports may be a hazard to the collectors. The actual cause of the saturations will be isolated as soon as possible.

V. SUMMARY AND STATUS

A. PROGRESS FOR THE YEAR

Gulliver III, developed during this contractual period, exhibits these new features: an omnidirectional capability, an antimetabolite injection system and a capability for flushing. All of these important developments have undergone field tests with satisfactory results.

There is now a better technique for deploying sample collection lines.

The temperature profile of the incubation chamber has been determined. The thermostatically controlled heating pad has performed properly in all tests and is considered a reasonably adequate means of incubation chamber temperature control for earth field testing.

Experiments with soil sample collection materials and techniques have indicated methods for increasing the amount of sample brought into the incubation chamber.

The requirements for flushing have been tentatively established (i. e., that a diffusion period at a reduced pressure is more effective than flushing and diffusion alone is probably adequate).

Several methods of gas collection were investigated, supporting the existing technique. Chemical reaction for gas fixation has been established as a preferable method to physical entrapment of the metabolic CO_2 by activated charcoal or molecular sieves.

Solid state detectors were investigated for feasibility.

Circuits for solid state detectors were designed; so if and when several advancements in the state of the art of these devices comes about they can be readily incorporated into the Gulliver instrument design.

Discussions with Amperex Corporation has resulted in their assurance that an anticoincidence geiger system can be produced in a short time which is capable of withstanding all of the environmental stresses specified for the flight instrument.

The overall feasibility of the automated life detection instrument has been more firmly established.

B. FUTURE DIRECTION

The problem area which we plan to pursue most vigorously in the near future is that of improving total test response. Efforts in this area will be made principally in three directions: (1) sample collection -- increasing the quantity of soil sample; (2) gas collection -- increasing the saturation limit of the collector compound by investigating different deposition methods and by considering compounds other than barium hydroxide; (3) improvement of counting efficiency by further investigation of the feasibility of detectors such as gas flow proportional counters.

Field testing will continue in order to demonstrate feasibility further, to gain reliability, and to test new techniques or improvements as they are incorporated in the design.

It is to be emphasized that extensive efforts in the areas of environmental testing and reliability improvement are required before theinstrument is ready for "flight." With the delay to 1966 for a Mars mission it is possible that some basic redesigns of the system can be incorporated before the intensive environmental and reliability testing effort is initiated; however these requirements have been and will continue to be considered in the earliest possible design stage.